INCREASED STRESS SUSCEPTIBILITY AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS DYSFUNCTION
EARLY MARKERS OF PSYCHOSIS VULNERABILITY?

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INCREASED STRESS SUSCEPTIBILITY AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS DYSFUNCTION: EARLY MARKERS OF PSYCHOSIS VULNERABILITY?

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ABSTRACT

Background: Individuals with psychosis are characterised by increased exposure and reactivity to psychosocial stressors and abnormal hypothalamic-pituitary-adrenal (HPA) axis function [i.e., elevated cortisol levels, a blunted cortisol awakening response (CAR), and pituitary volume abnormalities]. The extent to which these features are present among at-risk individuals, prior to illness onset, is currently unclear.

Aims: To determine whether putatively at-risk children who present multiple antecedents of schizophrenia (ASz) or a family history of illness (FHx) show increased susceptibility to psychosocial stressors and HPA axis abnormalities relative to typically-developing (TD) children. An additional aim was to explore associations between these measures and neurocognitive function.

Methods: ASz (n=35), FHx (n=25), and TD (n=44) children were identified at age 9-12 years using a novel community-screening procedure or as relatives of individuals with schizophrenia. Measures of psychosocial stress, salivary cortisol, pituitary volume, and neurocognitive function were obtained at age 11-14 years.

Results: Relative to TD children, both ASz and FHx children reported greater exposure to psychosocial stressors and were more distressed by these exposures (d=0.55-1.02, p<0.05). Additionally, FHx children, but not ASz children, showed a blunted CAR compared to TD children (d=0.73, p=0.01), yet neither group were characterised by elevated diurnal cortisol levels or pituitary volume abnormalities. In exploratory analyses, more abnormal HPA axis function (i.e., higher diurnal cortisol levels and a more blunted CAR) was associated with greater neurocognitive deficits among FHx and ASz children, whilst experiences of psychosocial stress were associated with better neurocognitive performance.

Conclusions: Increased stress susceptibility and a blunted CAR may constitute early markers of psychosis vulnerability whilst other HPA axis abnormalities (i.e., elevated diurnal cortisol levels and abnormal pituitary volume) may emerge more proximally to disease onset. Interventions to help at-risk youth cope more effectively with psychosocial stress might alleviate further HPA axis abnormalities and avert transition to psychosis.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
ASz	Antecedents of schizophrenia
CAR	Cortisol awakening response
CBT	Cognitive behavioural therapy
CI	Confidence interval
d	Standardised mean difference
DSM	Diagnostic and Statistical Manual of Mental Disorders
ESM	Experience sampling method
FHx	Family history of schizophrenia
FIGS	Family Interview for Genetic Studies
HC	Healthy control
HPA	Hypothalamic-pituitary-adrenal
Min	Minutes
MRI	Magnetic resonance imaging
N	Total sample size
n	Subgroup sample size
OR	Odds ratio
PLE	Psychotic-like experience
PTSD	Post-traumatic stress disorder
SD	Standard deviation
SE	Standard error
SPD	Schizotypal personality disorder
TD	Typically-developing
UHR	Ultra high-risk

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STATEMENT OF PERSONAL CONTRIBUTION

The Child Health and Development Study is a longitudinal investigation established in 2003 by Dr Kristin Laurens and Professors Sheilagh Hodgins, Barbara Maughan, Robin Murray, and Eric Taylor. With support from my supervisors, Dr Laurens and Professor Carmine Pariante, I developed a PhD proposal to examine psychosocial stress and HPA axis function within this cohort and obtained a studentship to complete the project. I also contributed to drafting the application for ethical approval to conduct the follow-up assessment phases of the study.

During the course of the study, I participated in the recruitment of children with a family history of schizophrenia who were identified via clinical records, and completed the Family Interview for Genetic Studies (FIGS) with 70 caregivers in total. With regards to the data examined in this thesis, I developed the protocol for collecting salivary cortisol samples with support from my supervisors, established and maintained all databases relating to this aspect of the project, and supervised undergraduate research placement students who coordinated saliva sample collection. I aliquoted and catalogued all saliva samples prior to their assay which was performed by Dr Patricia Zunszain at the James Black Centre, Institute of Psychiatry. Psychosocial stress measures were completed independently by participants under the supervision of placement students. I developed the scoring algorithm for the psychosocial stress measures, and scored all assessments examined in this thesis. I completed MRI scans with 69 of the 79 participants who contributed data to this thesis, and completed all manual tracing of the pituitary gland on these images. I additionally conducted 35 of the 99 neurocognitive assessments examined in this thesis. Finally, in discussion with my supervisors, I planned and conducted all statistical analyses and wrote this thesis in its entirety.

ASSOCIATED PUBLICATIONS

Publications relating to the work presented in this thesis

Cullen, A.E., Fisher, H.L., Roberts, R.E., Pariante, C.M., Laurens, K.R. (in press). Daily stressors and negative life events in children at elevated risk of developing schizophrenia. *The British Journal of Psychiatry*.

Cullen, A.E., Zunszain, P.A., Dickson, H., Roberts, R.E., Fisher, H.L., Pariante, C.M., Laurens, K.R. (submitted). Cortisol awakening response and diurnal cortisol levels among children at elevated risk for schizophrenia: Relationship to psychosocial stress and neurocognitive function. *Psychoneuroendocrinology*.

Related publications completed during the course of this PhD

Dickson, H., **Cullen, A.E.,** Reichenberg, A., Hodgins, S., Campbell, D.D., Morris, R.G., Laurens, K.R. (in press). Cognitive impairment among children at-risk for schizophrenia*. Journal of Psychiatric Research*.

Cullen, A.E., De Brito, S., Gregory, S., Williams, S.C.R., Murray, R.M., Hodgins, S., Laurens, K.R. (2013). Temporal lobe volume abnormalities precede the prodrome: A study of children presenting antecedents of schizophrenia. *Schizophrenia Bulletin*, *39,* 1318-1327.

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Related publications arising from MSc studies

Cullen, A.E., Dickson, H., West, S.A., Morris, R.G., Mould, G.L., Hodgins, S., Murray, R.M., Laurens, K.R. (2010). Neurocognitive performance in children aged 9-12 years who present putative antecedents of schizophrenia. *Schizophrenia Research 121,* 15-23.

INTRODUCTION

Aetiological theories of schizophrenia have been greatly influenced by the diathesis-stress model (Rosenthal, 1970; Zubin & Spring, 1977), which proposes that psychosocial stress can lead to the onset of psychosis among individuals with an underlying biological vulnerability for the disorder. In support of the model, major life events, for example the death of a loved one, have been associated with psychosis onset and relapse in clinical populations (Brown & Birley, 1968; Canton & Fraccon, 1985; Bebbington et al., 1993), and with the presence of psychotic symptoms in the general population (Johns et al., 2004; Lataster et al., 2012; van Nierop et al., 2012). Prospective studies of individuals with schizophrenia also indicate that minor daily hassles, such as those relating to work or family relations, are associated with illness relapse and symptom severity (Malla et al., 1990; Norman & Malla, 1994). Additionally, there has been considerable interest in the association between psychosis and traumatic experiences in childhood (e.g., physical and sexual abuse and neglect); with recent meta-analyses concluding that childhood trauma increases the risk for psychosis (Varese et al., 2012; Matheson et al., 2013a). Finally, there is evidence that stressful events elicit greater emotional reactivity in individuals with psychosis compared to healthy controls (Myin-Germeys et al., 2001).

Research conducted over the past four decades has thus provided evidence that psychosocial stressors contribute to the development and maintenance of psychosis. However, until recently, a biological explanation for how stress might act on the brain to give rise to the clinical and neurobiological features of schizophrenia was lacking. It has been proposed that the hypothalamic-pituitary-adrenal (HPA) axis, the primary system involved in coordinating the physiological response to stressors, may play a key role in mediating the relationship between stress and psychosis (Walker & Diforio, 1997; Walker et al., 2008).

In brief, the HPA axis responds to stress by triggering a cascade of hormonal reactions, which results in the secretion of glucocorticoids (primarily cortisol) from the adrenal cortex. Glucocorticoids interact with various systems throughout the body, allowing the organism to respond both behaviourally and physiologically to stress. These hormones also cross the blood-brain barrier and act on cells expressing glucocorticoid receptors (Krugers et al., 2012); by binding to these receptors in the hypothalamus and pituitary, glucocorticoids dampen HPA axis activity via a process of negative feedback (Laryea et al., 2013). Glucocorticoid receptors are also expressed in the hippocampus and medial prefrontal cortex, and both regions play a crucial role in mediating HPA axis function (Herman et al., 2005).

It is proposed that abnormal HPA axis function contributes to the clinical features of psychosis by augmenting the activity of dopamine (Walker et al., 2008), a neurotransmitter that has long been implicated in the pathogenesis of schizophrenia (Howes & Kapur, 2009). In support of this model, animal studies demonstrate that stressful experiences lead to increased synthesis and release of striatal dopamine (Howes & Murray, in press). Moreover, investigations in both animals and humans indicate that cortisol can enhance dopamine activity in certain brain regions, particularly the mesolimibic system (Walker et al., 2008). Thus, it is possible that the increase in presynaptic dopaminergic activity which has been consistently observed among individuals with schizophrenia (Howes et al., 2012) may be triggered by stress-induced HPA axis dysfunction.

Accumulated evidence indicates that schizophrenia is characterised by abnormal HPA axis function. Studies measuring cortisol in blood plasma have observed elevated cortisol levels in individuals with chronic schizophrenia (Christie et al., 1986; Gil-Ad et al., 1986; Whalley et al., 1989; Markianos et al., 1999; Muck-Seler et al., 2004; Zhang et al., 2005; Gallagher et al., 2007; Ritsner et al., 2007; Yilmaz et al.,

2007; Venkatasubramanian et al., 2010), and antipsychotic-naive patients experiencing their first psychotic episode (Abel et al., 1996; Ryan et al., 2003; Ryan et al., 2004; Spelman et al., 2007; Kale et al., 2010). More recently, studies have used repeated saliva sampling to assess the cortisol awakening response (CAR), which refers to the sharp increase in cortisol that typically occurs within 15-40 minutes of waking. Using this methodology, patients with first-episode psychosis have been found to show a blunted CAR relative to healthy controls (Mondelli et al., 2010a; Pruessner et al., 2013b). Studies using repeated salivary cortisol measures also demonstrate that individuals with schizophrenia show a blunted cortisol response during psychosocial stressor tasks (i.e., tasks designed to elicit an increase in cortisol) relative to healthy controls (Jansen et al., 2000; Brenner et al., 2009).

Whilst abnormal cortisol levels have also been observed among other psychiatric disorders, most notably, depression and post-traumatic stress disorder (PTSD), the specific patterns of abnormality that characterise these disorders are different to the pattern observed among individuals with psychosis. For example, patients with major depression typically show elevated diurnal cortisol levels (Stetler & Miller, 2011) and an increased CAR (Bhagwagar et al., 2003; Dienes et al., 2013; Ulrike et al., 2013), whilst PTSD is associated with decreased cortisol levels during the day (Morris et al., 2012) and a blunted CAR (Chida & Steptoe, 2009). Thus, by examining both the CAR and diurnal cortisol levels, it is possible to identify patterns of HPA axis abnormalities that distinguish between these disorders.

Further evidence that psychosis is associated with abnormal HPA axis function has been obtained in studies examining pituitary gland volume. Cross-sectional investigations have reported pituitary volume differences in patients with psychosis relative to healthy controls, with a divergent pattern of volume increases or decreases dependent on stage of illness. Typically, larger volumes have been

observed among medicated patients with first-episode psychosis relative to healthy individuals (Pariante et al., 2004; Pariante et al., 2005; Büschlen et al., 2011; Takahashi et al., 2011) while smaller volumes relative to healthy individuals have been reported among those with established schizophrenia (Pariante et al., 2004; Upadhyaya et al., 2007). However, other studies have observed no differences between first-episode patients and healthy controls (Nicolo et al., 2010; Gruner et al., 2012). Furthermore, longitudinal studies following individuals from the time of their first psychotic episode have observed pituitary volume increases (MacMaster et al., 2007a) and decreases (Nicolo et al., 2010) following antipsychotic treatment; although these inconsistent findings are likely to relate to differences in medication types across studies. The pituitary volume enlargements observed among antipsychotic-naive first-episode patients are thought to reflect an increase in the size and number of corticotroph cells that produce HPA axis hormones (Pariante, 2008). Although it is possible that the pituitary enlargements are due to increased secretion of other anterior pituitary gland hormones (e.g., prolactin, luteinising hormone, and follicle-stimulating hormone), these hormones have not been found to be elevated in first-episode populations relative to healthy controls (Pariante, 2008).

As well as giving rise to the clinical features of psychosis, stress-induced HPA axis function may also contribute to some of the neurocognitive impairments that characterise individuals with schizophrenia. Animal studies indicate that behavioural stressors and persistently-elevated glucocorticoid levels can lead to structural changes in the hippocampus and medial prefrontal cortex (Sapolsky, 2000; Cerqueira et al., 2008). Thus, the deficits in memory and executive function which have been consistently observed among individuals with schizophrenia (Reichenberg & Harvey, 2007), may, at least in part, be caused by HPA axis dysfunction triggered by psychosocial stress exposure. Indeed, among individuals with schizophrenia,

elevated cortisol levels have been correlated with poorer memory and executive function (Walder et al., 2000), and a study of individuals with first-episode psychosis observed that a more blunted CAR was associated with deficits in verbal memory (Aas et al., 2011b). Whether the observed associations between neurocognitive function and cortisol levels are indeed due to an excess of psychosocial stressors, however, is currently unclear. Whilst some studies of individuals with psychosis have reported that childhood maltreatment is associated with greater impairments in memory and executive function (Lysaker et al., 2001; Shannon et al., 2011), other studies examining the relationship between psychosocial stress exposure (including childhood maltreatment) and impairments in these specific domains have not (Schenkel et al., 2005; Aas et al., 2011b; Sideli et al., in press). Alternatively, abnormal neurodevelopmental processes (as opposed to psychosocial stress exposure) may influence the development of the brain regions that mediate both HPA axis function and these neurocognitive abilities.

The studies described above suggest that individuals with psychosis are characterised by increased exposure and reactivity to psychosocial stressors and a distinct pattern of HPA axis dysfunction relative to healthy individuals. Moreover, abnormal HPA axis function (possibly caused by psychosocial stress exposure) may be associated with some of the neurocognitive impairments observed among individuals with schizophrenia. However, it is currently unclear as to whether these HPA axis abnormalities emerge prior to the first psychotic episode and thus contribute to the development of psychosis, or whether they are merely a consequence of the stress associated with illness onset. The study of individuals at elevated risk for schizophrenia offers the opportunity determine the temporal relationship between psychosocial stress susceptibility (exposure and reactivity), HPA axis dysfunction, and psychosis.

Aims

This thesis examines experiences of psychosocial stress and HPA axis function in children aged 11-14 years with different vulnerability profiles for schizophrenia: those at putatively elevated risk who present psychotic-like experiences and other antecedents of the disorder, and those with a family history of illness. The primary aim was to determine whether these children are characterised by increased susceptibility to psychosocial stress (i.e., greater exposure to psychosocial stressors and increased reactivity to these exposures) and biological markers of HPA axis dysfunction (i.e., elevated diurnal cortisol levels, a blunted CAR, and pituitary volume abnormalities) relative to their typically-developing peers. Additionally, the current study aimed to examine the relationship between experiences of psychosocial stress and HPA axis function, and investigate the association between these measures and current symptoms of psychopathology. A final aim was to explore the extent to which neurocognitive function is associated with experiences of psychosocial stress and cortisol levels among children at elevated risk for schizophrenia.

Hypotheses

Based on previous studies examining individuals with schizophrenia (described above) and those at elevated risk for the disorder (reviewed in Chapter 2), the following hypotheses were tested:

With respect to experiences of psychosocial stress:

1a. Children at elevated risk for schizophrenia will be exposed to higher levels of psychosocial stressors than typically-developing children.

1b. Compared to typically-developing children, those at elevated risk for schizophrenia will be more distressed by psychosocial stressors.

1c. Experiences of psychosocial stress (exposure and reactivity) will be more strongly associated with current symptoms of psychopathology among high-risk children relative to typically-developing children.

With respect to cortisol indices of HPA function:

2a. Children at elevated risk for schizophrenia will show elevated diurnal cortisol levels and a blunted CAR relative to typically-developing children.

2b. Cortisol levels will be associated with exposure to psychosocial stressors and distress related to these exposures among high-risk children.

2c. Among children at elevated risk for schizophrenia, cortisol levels will be correlated with current symptoms of psychopathology.

With respect to pituitary volume indices of HPA function:

3a. Children at elevated risk for schizophrenia will show abnormal pituitary gland volume relative to typically-developing children.

3b. Among children at elevated risk for schizophrenia, pituitary volume will be correlated with salivary cortisol levels.

3c. Pituitary volume will be associated with exposure to psychosocial stressors and distress related to these exposures in high-risk children.

3d. No relationship between pituitary volume and current psychopathology will be observed among children at elevated risk for schizophrenia.

With respect to neurocognitive function:

4a. Experiences of psychosocial stress (exposure and reactivity) will be negatively associated with neurocognitive function in high-risk children.

4b. Among children at elevated risk for schizophrenia, more abnormal cortisol levels will be associated with poorer neurocognitive function.

THESIS OUTLINE

This thesis comprises eight chapters, a brief overview of each chapter is provided below.

Chapter 1 describes established strategies for identifying individuals at elevated risk for schizophrenia and outlines the rationale for developing a novel method to identify putatively high-risk children who present multiple antecedents of the disorder.

Chapter 2 comprises a systematic review of studies examining psychosocial stress, cortisol levels, and pituitary volume among individuals at elevated risk for schizophrenia. Methodological issues pertinent to the work presented in subsequent chapters are also discussed.

Chapter 3 describes the methodology of the overall study, with particular focus on the recruitment procedure and assessment phases. Details of the measures used to assess sociodemographic characteristics and current psychopathology are provided (primary measures described in subsequent chapters) and the analytic strategy employed in subsequent chapters is described.

Chapter 4 examines exposure to psychosocial stressors and distress relating to these exposures among children at elevated risk for schizophrenia and typically-developing children. This chapter also investigates whether the relationship between psychosocial stress and current psychopathology is associated with risk status.

Chapter 5 investigates the extent to which children at elevated risk for schizophrenia are characterised by abnormal cortisol levels (elevated diurnal cortisol and/or a blunted CAR) relative to their typically-developing peers. This chapter also examines the extent to which cortisol levels are associated with experiences of psychosocial stress and current psychopathology among high-risk children.

Chapter 6 compares pituitary gland volume in children at elevated risk for schizophrenia and typically-developing children. The relationship between pituitary volume and cortisol levels, experiences of psychosocial stress, and current psychopathology among high-risk children is also investigated.

Chapter 7 explores the extent to which experiences of psychosocial stress (exposure and reactivity) and more abnormal cortisol levels are associated poorer neurocognitive function among children at elevated risk for schizophrenia.

Chapter 8 reviews the main findings of the study and discusses these results in the context of the study hypotheses. This chapter also provides a detailed description of the methodological issues that are relevant to the overall study. The contribution of the work presented in this thesis to existing scientific knowledge is discussed, as are the implications of the findings for existing theories, clinical practice, and future research.

DEFINITION OF RESEARCH TERMS

Schizophrenia and psychosis

Schizophrenia is a severe mental disorder characterised by episodes of psychosis (i.e., a loss of contact with reality), often expressed as hallucinations, delusions, and disorganised speech and behaviour (American Psychiatric Association, 2013). Whilst there has been some controversy surrounding the diagnostic construct of schizophrenia (van Os, 2009; Keshavan et al., 2011; Tandon et al., 2013), the disorder has been retained, albeit with some modifications to the criteria, in the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders [DSM-5 (American Psychiatric Association, 2013)]. It is anticipated that next version of the International Classification of Disorders (ICD-11) will adopt a similar definition (Gaebel, 2012).

The DSM-5 criteria for schizophrenia are provided in Box 1. Although these criteria attempt to delineate schizophrenia from other psychotic disorders (e.g., schizoaffective disorder, schizophreniform disorder, delusional disorder, brief psychotic disorder, affective psychoses, and substance-induced psychotic disorder), the extent to which these can be considered separate diagnostic categories has been questioned (van Os, 2009). Despite calls from some experts to replace the categorical diagnosis with a more dimensional approach, this has not been implemented in the DSM-5 (Heckers et al., 2013). However, the new DSM incorporates eight dimensional scales which assess the severity of the five core schizophrenia symptoms (hallucinations, delusions, disorganised speech, abnormal psychomotor behaviour, and negative symptoms) in addition to impaired cognition, depression, and mania (Barch et al., 2013). It is hoped that these scales will be clinically useful, and that adopting a dimensional assessment approach will support research efforts to elucidate the causes of psychosis (Heckers et al., 2013).

Box 1. DSM-5 criteria for schizophrenia

Criterion A: Characteristic symptoms (two or more of the following, present for a significant portion of a one-month period, including at least one of symptoms 1-3):

1. Delusions
2. Hallucinations
3. Disorganised speech
4. Grossly disorganised or catatonic behaviour
5. Negative symptoms (i.e., diminished emotional expression or avolition)

Criterion B: Social/occupational dysfunction

Criterion C: Duration of at least six months

Criterion D: Schizoaffective and major mental disorder diagnoses excluded

Criterion E: Disturbance not attributed to substances or other medical condition

Criterion F: Relationship to global developmental disorder or autism spectrum disorder (i.e., if there is history of these disorders, an additional diagnosis of schizophrenia can only be made if prominent delusions or hallucinations are present for at least one month)

Limitations notwithstanding, the validity of schizophrenia as a construct is supported by the fact that several risk factors (described below) have been consistently associated with the disorder (Tandon et al., 2013). Furthermore, prospective studies of individuals who have recently experienced their first psychotic episode indicate that schizophrenia exhibits high diagnostic stability over time (Haahr et al., 2008; Bromet et al., 2011), whereas the stability of other psychotic disorders is far lower. As such, this thesis is concerned primarily with the extent to which schizophrenia specifically (and vulnerability for this disorder) is associated with experiences of psychosocial stress and HPA axis dysfunction[1].

[1] When describing the existing literature, the association between these factors and 'psychosis' is reported for studies that do not, or cannot, distinguish between specific psychotic disorders. For example, studies examining individuals who have recently experienced their first psychotic episode (herein referred to as individuals with 'first-episode psychosis') where the longitudinal course of illness and resultant diagnosis are not yet clear.

Schizophrenia is a relatively rare disorder, affecting approximately 0.4% of individuals during the course of their lifetime (Saha et al., 2005). Meta-analyses indicate that males are at greater risk for the disorder than females (McGrath et al., 2004), and there is evidence that illness onset (which typically occurs during late adolescence and early adulthood) is also slightly earlier in males (Eranti et al., 2013). Furthermore, in the UK specifically, the incidence is also higher among individuals of black Caribbean and black African ethnicity relative to the white British population (Kirkbride et al., 2012). Family studies indicate that schizophrenia is highly heritable (Sullivan et al., 2003); however, the genetic underpinnings of the disorder have yet to be elucidated as it seems that multiple genes are involved (Tandon et al., 2009). A number of environmental risk factors for schizophrenia have also been identified, including, prenatal infection, perinatal complications, urbanicity, migration, and cannabis use (Matheson et al., 2011). It is likely that these environmental factors may act in combination with genetic vulnerability to give rise to the disorder; indeed, several studies have identified gene-environment interactions which may act to increase the risk for schizophrenia (Modinos et al., 2013).

Despite advances in pharmacological and psychological treatments for schizophrenia, recent estimates indicate that only 1-2 individuals in every 100 experience recovery during the first year of illness (defined as improvement in both clinical symptoms and social functioning), with approximately 14% expected to recover over 10 years (Jaaskelainen et al., 2013). Of even greater concern is the fact that the risk of mortality is 2.5 times higher among individuals with schizophrenia compared to the general population (Saha et al., 2007), a trend which has worsened in recent decades. These findings emphasise the need to develop early identification and intervention strategies, which may help to avert illness onset among those who are at elevated risk for the disorder.

Antecedents of schizophrenia

This thesis examines children who may be at elevated risk for developing schizophrenia in later life because they present multiple antecedents of the disorder (Laurens et al., 2007; Laurens et al., 2011). Antecedents of schizophrenia are subtle developmental deviances which have been found to characterise individuals who later develop the disorder (Welham et al., 2009a). These characteristics are observable from infancy through to later life, and represent early expressions of schizophrenia. Thus, unlike other factors that have been associated with increased risk for the disorder (e.g., urbanicity, migration, and cannabis use), antecedents are on the aetiological pathway, and can be thought of as developmental precursors of later illness. Prospective longitudinal studies have identified a number of factors that may be considered childhood antecedents of schizophrenia, including, delays and abnormalities in speech development, motor dysfunction, social withdrawal and emotional problems, behavioural difficulties, intellectual and cognitive impairments, and psychotic-like experiences (Niemi et al., 2003; Welham et al., 2009a; Matheson et al., 2011; Dickson et al., 2012). It is thought that these antecedents are largely driven by genetic vulnerability factors, although environmental exposures may also contribute to their expression (Matheson et al., 2011).

The rationale for developing a novel strategy to identify children who present multiple antecedents of schizophrenia is described in detail in subsequent chapters. In brief, a novel community-screening procedure was used to identify children aged 9-12 years who present a triad of antecedents of schizophrenia (ASz) that could be assessed via questionnaire, including, (i) a speech and/or motor developmental delay or abnormality, (ii) a social, emotional, and/or behavioural problem, and (iii) a psychotic-like experience (Laurens et al., 2007). Throughout this thesis, the term 'ASz children' refers to children who present this triad of antecedents.

Psychosocial stress

Psychosocial stress is a broad concept that encompasses a wide range of experiences. In some studies, psychosocial stress refers to the specific exposures (or stressors) that are under examination (typically, events that are assumed to be stressful for the majority of individuals), whilst in other studies, the term is used to describe subjective feelings of 'stress' that result from these exposures (Cohen et al., 1995). Throughout this thesis, the terms 'exposure' and 'reactivity' are used to distinguish between these two measures of psychosocial stress. Thus, 'psychosocial stress exposure' is defined as the occurrence of a potentially stressful event, regardless of its actual impact, whilst 'psychosocial stress reactivity' refers to the distress or emotional reaction elicited in response to a psychosocial stressor. For brevity, the term 'experiences of psychosocial stress' is used to describe exposure and/or reactivity. Studies examining psychosocial stress exposures among individuals with schizophrenia have largely focused on major life events, minor daily hassles, and childhood trauma. These terms are defined as follows:

Major life events are negative or positive events, of infrequent occurrence, that would be considered by most individuals to be of major significance. Examples include, divorce or separation, changes to employment status, financial difficulties, bereavement, and moving home. The frequency and impact of these events may be assessed over the total life span or during a defined period of time (e.g., events occurring within the past year).

Minor daily hassles are common difficulties or challenges that occur throughout the course of daily life. Measures assessing daily hassles often include items relating to interpersonal relations (e.g., arguments with friends or family), work or school (e.g., problems with colleagues, peers, or teachers), and circumstance (e.g., transport problems or having to meet a deadline).

Childhood trauma is a broad term that is often used to refer specifically to experiences of childhood maltreatment (i.e., physical abuse, sexual abuse, neglect, and emotional abuse) but can also include other traumatic experiences that may occur in childhood, such as parental death or bullying.

The current study examines experiences of major negative life events and minor daily hassles (i.e., exposure to these psychosocial stressors and reactivity to these exposures) among children at elevated risk for schizophrenia. The study also explores the association between schizophrenia risk status and exposure to physical punishment (i.e., being slapped, spanked, or hit with an object), which is thought to lie on a continuum with childhood maltreatment and has been associated with schizotypal personality disorder (Afifi et al., 2012).

The hypothalamic-pituitary-adrenal (HPA) axis

In mammalian organisms, the physiological stress response is coordinated by two biological systems: (i) the autonomic nervous system, and (ii) the HPA axis (Day & Pariante, 2012; Krugers et al., 2012), both of which are regulated by the hypothalamus. The first system to respond is the sympathetic branch of the autonomic nervous system; activation of this system results in the rapid release of adrenaline which has immediate effects on heart rate, vasoconstriction, and digestion. If the threat is maintained the HPA axis is then activated, triggering a cascade of hormonal reactions (described in detail below). Of the two systems, the HPA axis has received particular attention from psychopathologists in light the fact that several psychiatric disorders (e.g., psychosis, depression, and PTSD) have been associated with HPA axis abnormalities (Walker et al., 2008).

The key components of the HPA axis are shown in Figure 1. In response to stressful stimuli, corticotrophin-releasing hormone (CRH) is secreted from the

paraventricular nucleus of the hypothalamus. CRH then triggers the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary which in turn stimulates the secretion of glucocorticoids (primarily cortisol) from the adrenal cortex. Glucocorticoids allow the organism to respond both behaviourally and physiologically to stress; these hormones interact with various systems throughout the body, influencing glucose metabolism, immune and cardiovascular function, and brain function (Walker et al., 2008). The brain expresses two forms of glucocorticoid receptor: (i) high-affinity mineralocorticoid receptors (MRs) which are occupied at rest, and (ii) low-affinity glucocorticoid receptors (GRs) that are typically occupied only under high glucocorticoid conditions (Krugers et al., 2012). Glucocorticoids regulate HPA axis activity by binding to these receptors in the hypothalamus and pituitary where they inhibit the secretion of CRH and ACTH respectively by a process of negative feedback (Laryea et al., 2013).

Other brain regions also play a crucial role in mediating HPA axis function. The hippocampus is densely populated with MRs (with specific regions expressing GRs), and also dampens HPA axis activity via negative feedback (Walker et al., 2008). There is also evidence that the medial prefrontal cortex and amygdala regulate HPA axis function (Herman et al., 2005; Cerqueira et al., 2008), although this complex process is not fully understood. Chronic stress and persistently-elevated glucocorticoid levels have damaging effects on the hippocampus, including, dendrite atrophy, suppression of long-term potentiation, inhibition of neurogenesis, and neuronal loss (Sapolsky, 2000; Corcoran et al., 2003). Moreover, it has been recently shown that behavioural stress can lead to structural changes in the medial prefrontal cortex of rodents (Cerqueira et al., 2008). Thus, chronic exposure to stress may have damaging effects on the key brain regions that mediate HPA axis function.

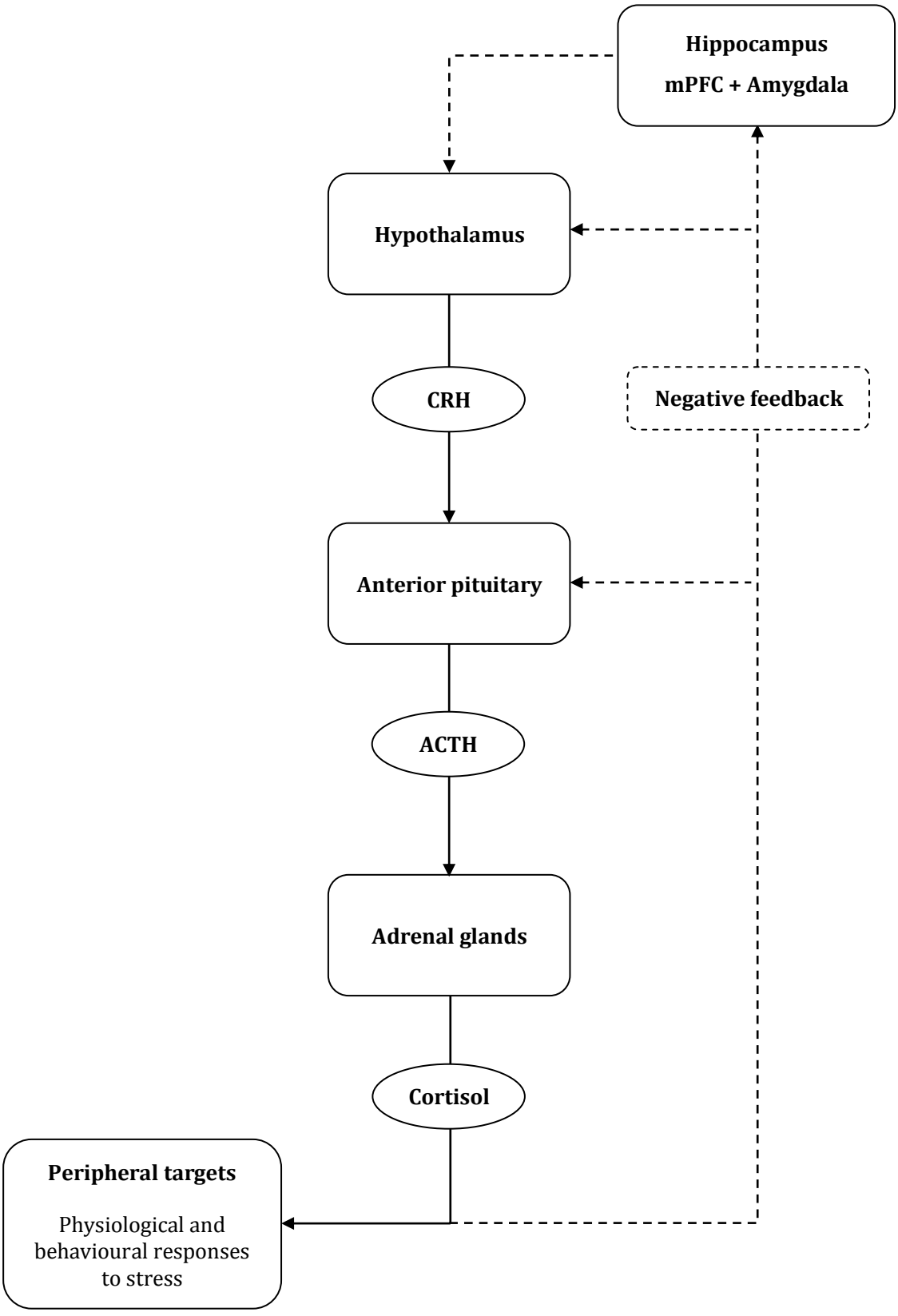

Figure 1. Key components of the HPA axis

Note. CRH: Corticotrophin-releasing hormone; ACTH: adrenocorticotropic hormone; mPFC: medial prefrontal cortex.

HPA axis activity can be inferred from levels of cortisol and ACTH in bodily fluids; cortisol can be reliably measured in blood, urine, and saliva, whilst ACTH can be measured only in blood. Given the invasiveness of obtaining blood samples and the distress associated with this procedure, salivary cortisol is now commonly used to assess HPA axis function in research settings (Walker et al., 2008). Cortisol levels can be examined under basal conditions, in response to awakening, and following exposure to psychosocial stress or pharmacological challenge (Day & Pariante, 2012). Basal cortisol levels can be estimated by obtaining a single sample (saliva, urine, or blood) during the day. However, as cortisol exhibits a distinct circadian rhythm (levels are highest in the morning, gradually decline throughout the day, and rise again during sleep) measurements are greatly affected by the time of sampling, and studies often obtain multiple samples throughout the day to examine diurnal patterns of secretion. More recently, there has been substantial interest in the cortisol awakening response (CAR), which describes the peak in cortisol levels that typically occurs within the first 15-40 minutes after awakening (Pruessner et al., 1997) and appears to be distinct from the circadian rhythm of cortisol (Fries et al., 2009). Changes in HPA axis activity in response to acute stress can be examined using psychosocial stressor tasks. These experimental procedures typically involve assessing cortisol levels before, during, and after a potentially stressful task (e.g., public speaking or mental arithmetic) in order to assess the change in cortisol secretion. Finally, HPA axis function can be assessed using the dexamethasone suppression test, which involves administering dexamethasone (a synthetic glucocorticoid) in order to determine whether the negative feedback system is effective in suppressing cortisol secretion (Corcoran et al., 2003).

Pituitary volume has also been used as a marker of HPA axis activity. Pituitary volume enlargements are thought to reflect an increase in the size and number of

corticotroph cells producing ACTH (Pariante, 2008), thus indicating increased HPA axis activity. Given that cortisol levels vary substantially throughout the day, it has been suggested that pituitary volume may provide a more stable measure of HPA axis function (Zipursky et al., 2011). Hypothalamic volume has also been employed as a measure of HPA axis function, albeit less commonly.

The current study examines three biological markers of HPA axis activity: (i) salivary cortisol levels throughout the day, (ii) the increase in salivary cortisol in response to awakening, and (iii) pituitary gland volume. In order to facilitate comparisons between the pattern of cortisol secretion observed among children at elevated risk for schizophrenia and that which characterises individuals with established illness, the saliva sampling procedure employed in the current study was based on the protocol used in previous studies of individuals with first-episode psychosis (Mondelli et al., 2010a; Aas et al., 2011b). Consistent with previous studies of first-episode patients (Mondelli et al., 2010a; Aas et al., 2011a; Pruessner et al., 2013b), cortisol data obtained at multiple time-points were then summarised using the two area under the curve (AUC) computations described by Pruessner et al. (2003). Similarly, the anatomical boundaries of the pituitary gland were determined using a protocol implemented in previous studies examining individuals with psychosis (Pariante et al., 2004; Pariante et al., 2005) and those at elevated risk for the disorder (Garner et al., 2005; Mondelli et al., 2008).

CHAPTER 1 Strategies for identifying individuals at elevated risk for schizophrenia

1.1 Introduction

In recent decades, there has been increased interest in developing strategies to identify individuals at elevated risk for schizophrenia (Yung & McGorry, 2007). There are two primary motivations for developing such methods. Firstly, a longer duration of untreated psychosis has been associated with more severe positive and negative symptoms, higher levels of global psychopathology, poorer social functioning, and higher rates of illness relapse and suicide (Marshall et al., 2005; Perkins et al., 2005; Farooq et al., 2009). These findings emphasise the importance of intervening early in the course of illness, if possible, even before the onset of psychosis. Secondly, the study of high-risk individuals offers the opportunity to identify disease characteristics that precede illness onset and contribute to the development of psychosis. The low base rate of schizophrenia in the general population requires that studies examining causal mechanisms in 'unselected' population cohorts assess large numbers of individuals, thereby imposing logistical limitations on the data that can be obtained. Samples enriched with individuals at high-risk for schizophrenia can be used to overcome the low base rate problem. These smaller 'high-risk' studies permit more complex and costly assessments (e.g., neuroimaging investigations) which provide important insights into the aetiology of schizophrenia.

The notion that it might be possible to premorbidly identify individuals who will later develop schizophrenia is predicated on the neurodevelopmental hypothesis (Murray & Lewis, 1987; Weinberger, 1987). In essence, the theory proposes that schizophrenia is caused by a combination of genetic and early environmental insults which lead to an abnormal pattern of neurodevelopment commencing in early life.

Studies conducted over the past 25 years have provided overwhelming support for the genetic and environmental contributions underlying the neurodevelopmental hypothesis. Family studies indicate that schizophrenia is a heritable disorder (Tandon et al., 2009) and subsequent advances in molecular biology have identified a range of genetic variations which confer susceptibility for the disorder (Allen et al., 2008; Schmidt-Kastner et al., 2012). In line with the suggestion that environmental factors operating in early life may contribute to schizophrenia, epidemiological studies have provided evidence that prenatal exposures (e.g. viral infections) and obstetric complications increase the risk for the disorder (Fatemi & Folsom, 2009). Prospective studies have also confirmed that subtle markers of abnormal neurodevelopment, for example, early developmental delays, cognitive impairments, and social, emotional, and behavioural problems, can be observed among children who later develop schizophrenia (Rapoport et al., 2005; Laurens et al., 2007). These findings indicate that it may be possible to identify individuals who are at increased risk for schizophrenia years before the onset of illness.

Chapter aims

The following chapter summarises existing strategies for identifying individuals at elevated risk for schizophrenia and describes a novel method that identifies children presenting multiple antecedents of the disorder. The specific aims were as follows:

1. Describe established strategies for identifying high-risk individuals and evaluate the strengths and limitations of these approaches.

2. Provide an overview of the clinical staging model of schizophrenia.

3. Outline the rationale for developing a novel to identify children at putatively elevated risk for schizophrenia who present multiple antecedents of the disorder and describe the characteristics of these children.

1.2 Established high-risk strategies

1.2.1 Individuals with a family history of illness

Background and rationale

Traditionally, the identification of individuals at elevated risk for developing schizophrenia has focused on those with a family history of illness. This approach is based on the understanding that schizophrenia is a heritable disorder, an observation first noted at the beginning of the twentieth century (McGuffin et al., 1995). Early studies reported that the risk of schizophrenia among offspring and siblings of patients with the disorder (i.e., first-degree relatives) is approximately 10 times higher than the risk in the general population (Tsuang et al., 2001). Whilst these studies provided initial support for the notion that genetic factors may play a role in the development of schizophrenia, they were not able to disentangle the effect of shared environments. Twin and adoption studies have provided more robust evidence of a genetic contribution to schizophrenia. These studies demonstrate that monozygotic twins show far higher concordance rates for schizophrenia than dizygotic twins (46% vs. 14%) (Gottesman & Shields, 1982), and that offspring of individuals with schizophrenia who are adopted by healthy individuals remain at elevated risk for the disorder (Tienari & Wynne, 1994). Pooled data from these studies has since shown the heritability of schizophrenia to be approximately 80% (Sullivan et al., 2003).

Genetic linkage studies and candidate gene approaches have led to the identification of several schizophrenia susceptibility genes, including, NRG1, DTNBP1, and COMT (Harrison & Owen, 2003). More recently, genome-wide association studies, capable of examining the entire genome, have been employed to identify genetic variants that distinguish between individuals with schizophrenia and healthy controls. These studies show that a range of common genetic variations of

small effect (odds ratios < 1.3) contribute to the risk of schizophrenia, as well as a number of relatively rare variants, known as copy number variations, which individually confer greater risks for the disorder (odds ratios range: 3-30) (van Winkel et al., 2010; Owen, 2012; Mowry & Gratten, 2013). Although the exact role of these genetic variants is currently unclear, a family history of illness remains one of the best-established risk factors for schizophrenia.

Characteristics of individuals with a family history of illness

Relatives of individuals with schizophrenia have been intensively studied over the past fifty years. Typically, these studies have focused on offspring of individuals with schizophrenia, following the development of these 'genetic high-risk' individuals from early life into adulthood. More recent cross-sectional studies have employed broader criteria for inclusion and have examined groups of siblings, parents, and second-degree relatives (e.g., grandparents, aunts and uncles, and half-siblings). Although the majority of individuals with a family history of illness will not go on to develop schizophrenia themselves, these individuals (regardless of their own illness outcome) share many characteristics with their affected relatives. There is consistent evidence that, relative to controls, individuals with a family history of illness are characterised by neuromotor abnormalities (Niemi et al., 2003), poorer performance on tests of cognitive function, particularly in the domains of verbal memory, executive function, and general intelligence (Sitskoorn et al., 2004; Agnew-Blais & Seidman, 2013), reduced grey matter in the anterior cingulate (Fusar-Poli et al., in press), altered brain activation, predominately in the temporal lobe (Cooper et al., in press), impaired psychosocial functioning (Owens & Johnstone, 2006), and deficits social cognition (Lavoie et al., 2013). The magnitude of differences between relatives and healthy controls on these measures is often smaller than the impairments observed among individuals with established illness.

Prediction of schizophrenia outcome

Whilst there are now greater than 16 prospective genetic high-risk studies established in countries throughout the world (Niemi et al., 2003; Owens & Johnstone, 2006), relatively few have followed individuals past the age of peak illness onset (~30 years) to determine the proportion who go on to develop illness. Summarising data from five studies which assessed schizophrenia outcome at follow-up, a recent study reported that between 5-26% of individuals with a family history of illness go on to develop psychosis (Goldstein et al., 2010). However, the elevated risk among those with a parental history of schizophrenia does not appear to be confined to psychotic disorders. A large population-based cohort study of individuals born in Denmark found that offspring of parents with non-affective psychosis were also at greater risk of developing bipolar disorder, depression, anxiety disorders, personality disorders, and substance misuse disorders (Dean et al., 2010). Among individuals with a family history of illness, those who later develop schizophrenia have been found to show more pronounced deficits in attention and verbal memory (Erlenmeyer-Kimling, 2000), social adjustment problems and behavioural difficulties (Niemi et al., 2003; Cannon & Clarke, 2005), neurological and neuromotor dysfunctions (Erlenmeyer-Kimling, 2000), and higher scores on psychosis-related personality scales (Carter et al., 1999; Bolinskey et al., 2001) relative to high-risk individuals who do not develop illness. As few genetic high-risk studies have prospectively followed individuals into adulthood, these factors may represent only a subset of those that confer additional vulnerability for schizophrenia.

Strengths and limitations

The genetic high-risk approach has been a highly successful research strategy which has helped to elucidate the role of genetic and environmental risk factors in the development of schizophrenia. One major advantage of the approach is that the

ability to identify individuals with a family history of illness in early life has enabled researchers to examine risk factors operating throughout childhood and adolescence. However, genetic high-risk studies have a number of limitations. Given that the majority of individuals with schizophrenia do not have a relative with the disorder (Gottesman & Shields, 1982; Mortensen et al., 2010), risk factors identified in these studies may relate to a more familial form of schizophrenia, which may not generalise to the majority of individuals who develop the disorder. In addition, many of the recent cross-sectional studies include relatives who have passed the age of peak risk for the development of illness (~30 years). Thus, factors distinguishing between these older relatives and healthy controls may not represent risk factors for illness onset, as by definition, these factors characterise those who have not developed schizophrenia (Cannon, 2005). Nonetheless, the family history approach is a useful strategy that enables the identification of disease characteristics that may be genetically-mediated.

1.2.2 Youth at ultra high-risk for psychosis

Background and rationale

A more recent strategy for identifying individuals at elevated risk for developing schizophrenia focuses on help-seeking adolescents and young adults who are thought to be in the prodromal phase of illness that immediately precedes the onset of florid psychosis (Yung & McGorry, 1996). The approach draws on retrospective observations published over several decades which show that many individuals with schizophrenia experience a prodromal period characterised by non-specific symptoms (e.g., reduced concentration and motivation, depression, anxiety, sleep disturbances, irritability, social withdrawal, and poor functioning) and/or subthreshold psychotic symptoms. The Personal Assessment and Crisis Evaluation (PACE) clinic was established in Melbourne, Australia to provide treatment for youth

presenting with features consistent with the prodrome (Yung et al., 1996). It was anticipated that this 'close-in' strategy would identify individuals who were more proximal to illness onset than the traditional genetic high-risk approach (McGorry et al., 2003). As the prodrome can only be determined retrospectively, and not all individuals will develop psychosis, the terms 'ultra high-risk' (UHR, or clinical high-risk in North American samples) and at-risk mental state (ARMS) have been used to describe those who present with potentially prodromal symptoms.

UHR status is typically assessed using the Comprehensive Assessment of At-Risk Mental States (CAARMS) which requires the presence of at least one of the following features: (i) attenuated (subthreshold) psychotic symptoms during the past year, (ii) brief, limited, or intermittent psychotic symptoms (BLIPS; i.e., frank psychotic symptoms of short duration that spontaneously remitted), or (iii) a family history of psychosis or diagnosis of schizotypal personality disorder in combination with a decrease in functioning (Yung et al., 2005). Early identification centres have now been established throughout the world. North American sites have tended to use the Structured Interview for Prodromal Symptoms and the accompanying Scale of Prodromal Symptoms [SIPS/SOPS (Miller et al., 2003)] to identify these at-risk youth, which employ the same criteria as the CAARMS. A related approach, which aims to identify individuals earlier in the prodromal stage of illness than the UHR strategy, focuses on those presenting 'basic symptoms', defined as subjectively identified disturbances of thought processing, perception, language, and attention (Klosterkotter et al., 1996; Klosterkotter et al., 2001). Basic symptoms can be determined using the Bonn Scale for the Assessment of Basic Symptoms and the Schizophrenia Proneness Instrument (Schultze-Lutter et al., 2007) and are often assessed in conjunction with UHR criteria (Fusar-Poli et al., 2013).

Characteristics of UHR youth

Recent meta-analyses indicate that UHR youth present neurocognitive impairments across a range of domains, including, general intelligence, memory, language function, attention, and executive function (Fusar-Poli et al., 2012b; Giuliano et al., 2012). These deficits are typically small-to-moderate in magnitude (that is, smaller than those observed in patients with established illness but similar in magnitude to those seen in individuals with a family history of illness). Whilst the larger of these meta-analyses indicated that UHR youth are also characterised by moderate impairments in social cognition (Fusar-Poli et al., 2012b), a further review found mixed evidence for the presence of these deficits among UHR youth (Thompson et al., 2011b). Structural brain abnormalities have also been examined extensively in this population. A recent meta-analysis, which included data from studies examining UHR youth and individuals reporting basic symptoms, observed reduced grey matter in the right middle and superior temporal gyri, the left anterior cingulate, and the right middle frontal gyrus in these youth relative to healthy controls (Fusar-Poli et al., 2012c). In line with the suggestion that the basic symptom approach may capture individuals in an earlier phase of the prodrome than UHR criteria, comparatively smaller deficits in neurocognitive functioning (Frommann et al., 2011) and less widespread structural brain abnormalities (Koutsouleris et al., 2009) have been observed in youth experiencing basic symptoms. Studies also indicate that UHR youth are characterised by significant impairments in social and role functioning (Cornblatt et al., 2007a; Shim et al., 2008; Carrion et al., 2011; Hui et al., 2013) which have been associated with more pronounced neurocognitive impairments (Niendam et al., 2007; Carrion et al., 2011; Lin et al., 2011). There is also some evidence to suggest that, like individuals with schizophrenia, UHR youth show elevated striatal dopamine relative to healthy controls (Howes et al., 2009); however, replication of this finding is needed.

Prediction of schizophrenia outcome

There has been considerable interest in the extent to which the UHR 'close-in' strategy identifies individuals at imminent risk of transitioning to psychosis. Early data from the original PACE clinic indicated that 41% of those meeting UHR criteria developed psychosis within one year (Yung et al., 2003). Similarly, a 12-month transition rate of 54% was reported in an early North American cohort of UHR youth (Miller et al., 2002). However, subsequent studies of UHR youth have reported lower transition rates. A review of studies from the PACE clinic found that the transition rate declined with each successive year, which was partially explained by the fact that UHR youth examined in more recent studies had experienced a shorter duration of symptoms prior to receiving help (Yung et al., 2007). This suggests that UHR youth are being detected and treated at an earlier stage of illness. A recent meta-analysis of 27 studies from a range of clinics observed transition rates of 18% at six months, 22% at one year, 29% at two years, and 36% at three years (Fusar-Poli et al., 2012a), and confirmed that more recent studies reported lower transition rates.

A range of factors have been associated with transition to psychosis among UHR youth, including, a longer duration of symptoms prior to identification, social functioning deficits, genetic risk and functional decline, higher levels of suspicion and paranoia, unusual thought content, substance use, and negative symptoms (Cannon et al., 2008; Thompson et al., 2011a; Nelson et al., 2013). Those who transition to psychosis have also been found to show more pronounced deficits in neurocognitive function (Fusar-Poli et al., 2012b; Giuliano et al., 2012), and reduced grey matter volume in the temporal lobe, cingulate, insular, and prefrontal cortex (Smieskova et al., 2010) compared to UHR individuals who do not. Observational data indicates that UHR youth receiving antidepressants are less likely to develop psychosis (Cornblatt et al., 2007b; Fusar-Poli et al., 2007). However, randomised trials investigating the

extent to which antipsychotic medications and/or cognitive behavioural therapy (CBT) can reduce transition rates have provided mixed evidence (McGorry et al., 2002; Morrison et al., 2004; McGlashan et al., 2006; Addington et al., 2011b). A meta-analysis of 11 trials reported that there was moderate quality evidence for the ability of CBT to reduce transition at 12 months (but not at 18 months), low quality evidence for the effects of omega-3 fatty acids, and no reported benefits for antipsychotic medications (Stafford et al., 2013). Thus, the extent to which current treatments can successfully delay or avert transition to psychosis among UHR youth is unclear.

Strengths and limitations

The success of the UHR approach cannot be disputed; over past 15 years high-risk centres have been established in countries throughout the world and 'attenuated psychosis syndrome' was recently considered for inclusion in the DSM-5 (Tsuang et al., 2013). This 'close-in' strategy has led to the identification of individuals who are much closer to the point to illness onset than those identified solely on the basis of a family history of schizophrenia. Studies of UHR youth therefore offer the opportunity to identify factors that may directly trigger the onset of psychosis. Furthermore, the results of these studies may be more generalisable than those obtained in genetic high-risk studies, which possibly relate only to a familial form of illness. However, a substantial proportion of UHR youth are treated with antipsychotics and other psychotropic medications (Woods et al., 2013), which are known to affect both brain structure and function. Thus, characterising premorbid neuroanatomical and neurocognitive abnormalities in UHR samples is confounded by treatment effects.

From a clinical perspective, UHR youth already present with high levels of impairment and disability, to the extent that they are sufficiently distressed as to seek help for their symptoms. There is also evidence that even those who do not convert to psychosis continue to show poorer functioning (Addington et al., 2011a),

implying that the UHR state itself can be regarded as a negative outcome. Furthermore, the extent to which treatments such as antipsychotic medications and CBT can avert transition to psychosis is currently unclear (Stafford et al., 2013). Thus, the identification of individuals at elevated risk for psychosis at an earlier stage of illness (that is, prior to the emergence of features consistent with the prodromal phase) may help to reduce the negative outcomes associated with UHR status.

1.2.3 Individuals with schizotypal personality disorder

Background and rationale

Schizotypal personality disorder (SPD) is a disorder within the schizophrenia spectrum that is characterised by attenuated forms of many of the traits and symptoms that define schizophrenia. Whilst SPD symptoms can be distressing and pervasive, unlike those of schizophrenia, they do not typically confer the need for long-term care (Chemerinski et al., 2013). The disorder originated from the observation that relatives of patients with schizophrenia are often characterised by schizophrenia-like traits, leading to the development of the term 'schizotype' to describe the inherited schizophrenia phenotype (Rado, 1953; Meehl, 1962). SPD was subsequently included in DSM III and in the current DSM version requires the presence of at least five of the following traits: (i) ideas of reference, (ii) odd beliefs or magical thinking, (iii) unusual perceptual experiences, (iv) odd thinking and speech, (v) suspiciousness or paranoid ideation, (vi) inappropriate or constricted affect, (vii) odd behaviour or appearance, (viii) the absence of close friends, and (ix) excessive social anxiety associated with paranoia (American Psychiatric Association, 2013). Several scales have been developed to measure schizotypal traits in the general population; the most widely employed being the Chapman scales (Chapman et al., 1995), which assess perceptual aberration, magical ideation, physical anhedonia, and social anhedonia, and the Schizotypal Personality Questionnaire

(Raine, 1991), which maps more closely to the DSM criteria. The prevalence of SPD in the general population ranges from 0.6% to 4.6% (Raine, 2006), and, as in schizophrenia, rates are higher among males (Pulay et al., 2009). In line with original conceptions of schizotypy, family and adoption studies concur in showing that SPD is more prevalent among relatives of individuals with schizophrenia than healthy controls (Siever & Davis, 2004), and that relatives also show higher scores on scales assessing social-interpersonal schizotypal symptoms (Tarbox & Pogue-Geile, 2011).

Characteristics of individuals with SPD

Individuals with SPD appear to present some, but not all, of the neurobiological features associated with schizophrenia. Psychophysiological abnormalities, including, reduced P50 suppression, abnormal startle blink reflex, reduced P300 event-related brain potentials, and impaired smooth-pursuit eye movements have been observed among individuals with SPD and members of the general population with schizotypal traits (i.e., individuals who obtain high scores on schizotypy measures but who do not meet SPD criteria) (Siever & Davis, 2004; Raine, 2006). Compared to healthy controls, individuals with SPD also show poorer performance on measures of working memory, verbal memory, and executive functioning (Siever & Davis, 2004; Seeber & Cadenhead, 2005; Raine, 2006), although IQ appears to be relatively intact. Similarly, whilst structural brain abnormalities encompassing the superior temporal gyrus, corpus callosum, and thalamus have been found among individuals with SPD (Dickey et al., 2002; Hazlett et al., 2012), grey matter reductions in the frontal regions and medial temporal lobe have not. Finally, there is evidence that individuals with SPD also display higher rates of dyskinetic movement abnormalities than healthy controls (Walker et al., 1999; Mittal et al., 2007). Such findings may be indicative of abnormal function of the dopamine system, a suggestion which is partially supported by neurochemical imaging studies of individuals with SPD (Siever & Davis, 2004).

Prediction of schizophrenia outcome

Few studies have followed individuals with SPD longitudinally to determine psychosis transition rates in this population. One early study found that 40% of inpatients with SPD developed schizophrenia within a 15-year follow-up (Fenton & McGlashan, 1989), where lower IQ, poorer work functioning, and transient delusion symptoms at baseline were associated with increased risk of transition to psychosis. More recently, transition rates of 25% and 48% were reported among psychiatric patients with SPD receiving integrated and standard treatment, respectively (Nordentoft et al., 2006). The extent to which the results obtained in these clinical samples can be generalised to individuals with SPD in the general population is unclear. However, a similar transition rate (36% over 2.5 years) was reported for the subgroup of participants in the North American Prodrome Longitudinal Study who met SPD criteria only (Woods et al., 2009), comprising both help-seeking youth and individuals recruited from the community. Lower transition rates have been reported among university students with schizotypal features: Over a ten year follow-up period, youth who obtained high scores on the Chapman perceptual aberration and magical ideation scales showed elevated rates of psychosis compared to the control group (5% vs. 1%), although high scores on the non-conformity and physical anhedonia scales were not associated with greater risk (Chapman et al., 1994).

Strengths and limitations

The study of individuals with SPD who present attenuated features of schizophrenia has provided additional important insights into the aetiology of the disorder. However, by definition, these individuals present symptoms of sufficient severity to meet criteria for a schizophrenia spectrum disorder, which in some instances may warrant hospitalisation and treatment with antipsychotic medications. Whilst there is evidence that pharmacological agents can lead to improvements in symptoms and

cognitive function in SPD populations (Raine, 2006), identifying high-risk youth at an earlier stage of illness may prevent the need for such treatments. As a related issue, some studies of individuals with SPD have examined hospitalised patients whilst other studies have used advertisements to recruit non help-seeking members of the general population who meet SPD criteria at interview, this makes it difficult to compare findings across studies. An alternative approach has been to study youth with schizotypal traits (i.e., those who obtain high scores on measures of schizotypy) who do not meet full SPD criteria, although it is not clear that such individuals are indeed at greater risk of developing schizophrenia.

1.2.4 Individuals presenting psychotic-like experiences

Background and rationale

Schizophrenia has traditionally been viewed as categorical entity, characterised by features which are distinct from the experiences of healthy individuals. However, evidence emerging over the past decade has indicated that psychosis exists on a continuum of severity, with members of the general population exhibiting symptoms that are similar, albeit less severe, than those presented by individuals with schizophrenia (van Os et al., 2000; van Os, 2009; van Os & Linscott, 2012). These subclinical psychotic symptoms, or psychotic-like experiences (PLEs), show aetiological continuity with schizophrenia (van Os et al., 2009) and are thought to be an expression of the underlying distribution of schizophrenia liability in the population (van Os, 2009).

PLEs are common in the general population, with a median prevalence rate of around 7% (Linscott & van Os, 2012). However, estimates vary depending on the type of assessment method employed. Interview-based methods, such as the Composite International Diagnostic Interview [CIDI (Kessler & Ustun, 2004)] and the Schedule for Affective Disorders and Schizophrenia for School-Aged Children

[K-SADS (Kaufman et al., 1997)], are considered the gold standard. Self-report questionnaire measures offer the opportunity to screen large samples (Konings et al., 2006; Laurens et al., 2007; Kelleher et al., 2011), but are associated with much higher levels of PLE endorsement. However, these measures have been found to show adequate predictive validity with PLEs assessed at interview (Konings et al., 2006; Kelleher et al., 2011). Auditory hallucinations are prevalent among younger children (median prevalence ~17%), but appear to be less common in adolescent samples (median prevalence ~8%) (Kelleher et al., 2012a). Indeed, longitudinal studies indicate that the majority of PLEs experienced during childhood and early adolescence are transient in nature (De Loore et al., 2011; Dominguez et al., 2011; Thapar et al., 2012; Downs et al., 2013).

Characteristics of individuals presenting PLEs

The high prevalence of PLEs in child and adolescent populations has generated much interest regarding the clinical significance of these experiences. Children and adolescents who report PLEs are characterised by higher levels of emotional symptoms and behavioural problems than those who do not (Laurens et al., 2007; Scott et al., 2009; Armando et al., 2010; Polanczyk et al., 2010; Barragan et al., 2011; Kelleher et al., 2012b), and are more likely to display self-harm and suicidal behaviours (Nishida et al., 2010; Polanczyk et al., 2010; Kelleher et al., 2013b). Longitudinal studies show that adolescents who experience persisting PLEs, as opposed to those whose PLEs remit, are at greater risk of developing internalising and externalising psychopathology (De Loore et al., 2011; Mackie et al., 2011; Downs et al., 2013). There is also evidence that children reporting PLEs are characterised by some of the neurocognitive features observed among adults with established schizophrenia. For example, children who report PLEs have been found to show poorer linguistic abilities, deficits in processing speed, and attentional impairments

compared to healthy youth (Kim et al., 2012; Hameed et al., 2013; Kelleher et al., 2013a). Additionally, a recent neuroimaging study observed increased grey matter volume in the angular gyrus, orbitofrontal gyrus, and the superior and middle temporal gyri, as well as decreased grey matter volume in the inferior temporal gyrus in children reporting PLEs relative to their healthy peers (Jacobson et al., 2010).

Prediction of schizophrenia outcome

Data from the prospective, longitudinal investigation of a Dunedin birth cohort provided the first robust evidence that individuals reporting PLEs are at increased risk for psychotic disorders (Poulton et al., 2000). Children reporting 'weak' PLEs at interview (i.e., who responded 'yes, likely' on at least one PLE item among five) were five times more likely to meet criteria for schizophreniform disorder at age 26 years than children who did not report PLEs. Moreover, children reporting 'strong' PLEs (i.e., who responded 'yes, likely' on at least two PLE items or 'yes, definitely' on at least one item) experienced a 16-fold increase in risk. Importantly, PLEs were not found to predict mania or depression, suggesting some degree of specificity. A recent follow-up of the Dunedin cohort at age 38 years observed that the risk of developing schizophrenia was seven times higher among those who reported strong PLEs in childhood (Fisher et al., 2013a); whilst PLEs also increased the risk for post-traumatic stress disorder by three-fold, they were not associated with increased risk for other psychiatric disorders.

Similar findings have been obtained in other cohorts, although only one has used hospital admission (as opposed to research diagnostic criteria) to assess outcome (Werbeloff et al., 2012). This longitudinal study examined young adults (aged 25-34 years at study commencement) over a mean follow-up period of 24 years, and found that the risk of later hospitalisation for non-affective psychotic disorders was approximately four times higher among those who reported at least one PLE at

interview compared to individuals who did not report PLEs. Risks were further increased among those who were also characterised by poor social functioning or anxiety disorder.

Pooling data from six cohort studies, a recent meta-analysis calculated that the annual risk of developing a psychotic disorder is 3.5 times higher among individuals reporting PLEs compared to those who do not (Kaymaz et al., 2012). This meta-analysis also found evidence of a dose-response relationship between PLEs and psychotic disorders (i.e., more severe/persistent PLEs associated with greater risk), and although PLEs also increased the risk for non-psychotic disorders, these associations were weaker.

Strengths and limitations

The study of individuals presenting PLEs has several advantages over more established high-risk strategies. PLEs can be assessed with ease using self-report questionnaires, allowing that the association with relatively rare risk factors can be examined in large population-based cohorts. Additionally, although PLEs are associated with high levels of comorbid psychopathology and distress, they do not typically confer a need for care. Thus, studies of individuals with PLEs are not confounded by help-seeking behaviour or treatment with antipsychotic medication. However, PLEs are experienced by a far higher proportion of the general population than clinical psychosis, and in the vast majority of instances these experiences are transient and benign. Further research is needed to determine the mechanisms by which some PLEs become pathological and increase the risk for schizophrenia.

1.2.5 Summary of established high-risk approaches

Table 1 summarises established strategies for identifying individuals at elevated risk for schizophrenia with particular emphasis on the strengths and limitations associated with each approach. Broadly speaking, these approaches have focused on two groups of individuals: those at elevated genetic-risk, as conferred by a family history of illness, and those at symptomatic-risk due to the presence of clinical features (i.e., UHR youth, individuals with SPD, and those reporting PLEs). Given that the majority of individuals with schizophrenia do not have an affected relative with the disorder, strategies based on the latter approach may have increased generalisability over the traditional genetic high-risk method. However, the degree of distress and illness severity among individuals at UHR and those with SPD limits the extent to which premorbid features can be examined in these populations.

It is important to note that the distinction between these two broad approaches (i.e., genetic- vs. symptomatic-risk definitions) may be somewhat arbitrary. Firstly, the groups identified using these methods are by no means mutually exclusive. Indeed, one of the UHR criteria is the presence of a family history of illness in combination with a decline in function; thus, a subset of those meeting UHR criteria are also at genetic high-risk for schizophrenia. Similarly, relatives of individuals with schizophrenia are more likely to meet SPD criteria than members of the general population, which is not surprising given the origins of the term 'schizotype'. Secondly, the clinical and neurobiological features that characterise individuals at genetic-risk and those at symptomatic-risk are not necessarily the product of different mechanisms. Characteristics of genetic high-risk individuals may be driven by environmental exposures rather than genetically-mediated effects, whilst individuals at symptomatic-risk may possess any number of the genetic variants which have been associated with increased risk for schizophrenia.

Table 1. Summary of existing strategies for identifying individuals at elevated risk for schizophrenia

Group	Identification	Strengths	Limitations
Family history of schizophrenia	Relatives (typically offspring or siblings) of patients with psychosis or members of the general population recruited via advertisements	(i) Individuals can be identified in early life and followed longitudinally, (ii) provides the opportunity to identify psychosis vulnerability factors that may potentially be genetically-mediated	(i) The majority of individuals with schizophrenia do not have an affected relative, thus, findings may not generalise, (ii) many studies include individuals who have passed the peak age of illness onset (~30 years)
Ultra high-risk (UHR)	Help-seeking adolescents and young adults who present with clinical characteristics consistent with the prodromal phase of illness	(i) Close-in strategy identifies individuals who are more proximal to disease onset, (ii) findings from studies of UHR youth may be more generalisable than those of family history (genetic) high-risk studies	(i) Help-seeking population: UHR youth are already characterised high levels of disability and impairment, (ii) studies examining brain structure and cognitive function may be confounded by medication use
Schizotypal personality disorder (SPD)	Psychiatric service users or members of the general population recruited via advertisements who meet SPD criteria at interview or obtain high scores on schizotypy measures	(i) Not typically help-seeking (although sometimes includes treated samples), (ii) findings from studies of individuals with SPD may be more generalisable than those of family history (genetic) high-risk studies	(i) Individuals with SPD present symptoms of sufficient severity to warrant a schizophrenia spectrum disorder diagnosis, (ii) considerable heterogeneity in illness severity across studies
Psychotic-like experiences (PLEs)	Members of the general population who report psychotic-like experiences as assessed at interview or using self-report questionnaires	(i) Non help-seeking population, not confounded by medication use (ii) epidemiological approach, associations can be examined in general population samples	(i) High prevalence of PLEs indicates this approach may be too broad and that the majority of individuals identified will not go on to develop psychosis

1.3 Clinical staging model of schizophrenia

The study of individuals at elevated risk for psychosis has demonstrated that many of the clinical and neurobiological features that characterise individuals with schizophrenia are present to some degree before illness onset. Such findings are consistent with the clinical staging model of schizophrenia (McGorry et al., 2006; Yung & McGorry, 2007; Wood et al., 2011), which offers a framework by which to better characterise the disorder. Clinical staging, a method commonly implemented in other medical disciplines, attempts to define the extent to which the illness has progressed and thus where an individual lies on the continuum of severity. The strategy is based on two main assumptions, (i) individuals in the early stages of illness will show less severe symptoms and should respond better to treatment, and (ii) in accordance with risk-benefit principles, treatments offered at an early stage should be benign and more effective.

Drawing on the findings of several decades of high-risk research, the model outlines a series of stages that precede psychosis onset, each corresponding to the extent, progression, and impact of the illness. According to the model (Table 2), the early stages of psychosis are characterised by relatively mild or non-specific symptoms (e.g., depression and anxiety, neurocognitive deficits, or PLEs with mild functional impairment). For some individuals, these symptoms worsen, new symptoms develop, and progressive neuroanatomical and neurocognitive changes occur which eventually manifests as psychosis (Wood et al., 2011). Whilst chronic illness may follow, an important feature of the model is that not all individuals will progress to psychosis. It is hoped that the staging model will help to elucidate the disease process and ultimately lead to more successful intervention strategies. The approach may also help to distinguish between biological processes that contribute to disease onset and those that represent epiphenomena (McGorry et al., 2006).

Table 2. Clinical staging model of schizophrenia, adapted from McGorry et al. (2006) and Wood et al. (2011)

Stage	Description	Example populations	Proposed interventions
Stage 0	Increased risk of psychotic or severe mood disorder, no current symptoms	First-degree adolescent relatives of individuals with schizophrenia	Improved mental health literacy, family education, substance use education, brief cognitive skills training
Stage 1a	Mild or non-specific symptoms (neurocognitive deficits, PLEs with mild functional decline, mood or anxiety symptoms resulting in distress and/or help-seeking) with mild functional change or decline	Referrals from youth mental health services, primary care, or school counsellors	Formal mental health literacy, family psychoeducation, formal CBT, active substance abuse reduction
Stage 1b	Ultra high-risk (moderate but subthreshold symptoms) with/without mild to moderate neurocognitive changes, comorbid substance abuse, and functional decline	Help-seeking youth meeting CAARMS criteria	Family psychoeducation, formal CBT, active substance abuse reduction, atypical antipsychotic agents, antidepressants, or mood stabilisers
Stage 2	Full threshold disorder with moderate-to-severe symptoms, neurocognitive deficits, and functional decline	Individuals meeting psychosis criteria as defined by the CAARMS	Family psychoeducation, formal CBT, active substance abuse reduction, atypical antipsychotic agents, antidepressants or mood stabilisers, and vocational training
Stage 3	Incomplete remission or recurrence/relapse	Individuals with a recurrence of Stage 2 psychosis	As for Stage 2, with additional emphasis on medical and psychosocial strategies to achieve full remission
Stage 4	Severe, persistent, and unremitting illness	Patients with established schizophrenia and functional impairment	As for Stage 3, with additional emphasis on relapse prevention strategies and identifying early warning signs

Note. CAARMS: Comprehensive Assessment of At-Risk Mental States (Yung et al., 2005); CBT: cognitive behavioural therapy.

1.4 Children presenting multiple antecedents of schizophrenia

1.4.1 A novel high-risk strategy

Rationale

Researchers at the Institute of Psychiatry sought to develop a novel method for identifying putatively high-risk children in the general population who present multiple antecedents of schizophrenia (Laurens et al., 2007; Laurens et al., 2011). This strategy was designed to enable the identification of high-risk youth at an earlier stage of illness than the UHR approach in the hope that this would maximise opportunities for early intervention. A further aim was to develop a high-risk strategy that would support research efforts to identify causal processes contributing to schizophrenia. It was anticipated that the identification of high-risk children from community samples would minimise the role of confounding factors that influence the study of UHR youth (e.g., help-seeking behaviour and medication use) and address the generalisability issues associated with genetic high-risk studies.

Antecedents of schizophrenia

Prospective longitudinal studies of population cohorts and genetic high-risk studies have identified a number of factors that may be considered childhood antecedents of schizophrenia (i.e., factors that characterise children who later develop the disorder which may be early expressions of illness). These include: delays and abnormalities in speech development, motor dysfunction, social withdrawal and emotional problems, behavioural difficulties, intellectual and cognitive impairments, and psychotic-like experiences (Niemi et al., 2003; Welham et al., 2009a; Matheson et al., 2011). As these antecedents are typically non-specific (i.e., conferring risk for a range of mental health problems in adulthood), and the effect sizes associated with each individual antecedent are typically small, it was reasoned that identifying children

who present multiple replicated antecedents, rather than any single risk factor (e.g. PLEs), would provide a more specific and sensitive means of identifying putatively at-risk children. Given the low prevalence of schizophrenia in the general population, it was anticipated that large numbers of children would need to be screened in order to identify those presenting multiple antecedents. The selection of antecedents was therefore restricted to those that could be easily assessed via questionnaire.

Questionnaires were subsequently developed to identify children aged 9-12 years presenting a triad of antecedents of schizophrenia (ASz), defined as (i) a speech and/or motor developmental delay or abnormality, (ii) a social, emotional, and/or behavioural problem, and (iii) a psychotic-like experience. Longitudinal studies have provided robust evidence that these factors represent early antecedents of schizophrenia (see Table 3 for examples). Birth cohort studies indicate that children who later develop schizophrenia display markers of abnormal neurodevelopment; specifically, these individuals show delays in attaining speech and motor milestones, poorer expressive and receptive language skills, and motor coordination problems in early life (Jones et al., 1994; Bearden et al., 2000; Rosso et al., 2000; Isohanni et al., 2001; Cannon et al., 2002; Sørensen et al., 2010; Welham et al., 2010; McNeil et al., 2011). There is also consistent evidence from population-based cohort studies and high-risk studies that those who later develop schizophrenia are characterised by social withdrawal and emotional problems in childhood (Jones et al., 1994; Crow et al., 1995; Bearden et al., 2000; Cannon et al., 2002; Kim-Cohen et al., 2003; Schiffman et al., 2004) as well as externalising symptoms and behavioural difficulties (Amminger et al., 1999; Bearden et al., 2000; Cannon et al., 2002; Carter et al., 2002; Kim-Cohen et al., 2003; Welham et al., 2009b). Finally, children reporting PLEs are at increased risk for later development of schizophrenia and related disorders (Poulton et al., 2000; Welham et al., 2009b; Fisher et al., 2013a; Zammit et al., 2013).

Table 3. Examples of longitudinal studies identifying childhood antecedents of schizophrenia

Study	Sample	Risk factor	Method	Relationship to schizophrenia outcome
Speech and motor delays and abnormalities				
Jones et al. (1994)	British 1946 Birth Cohort (N=4,746)	Speech problems	Clinical observations at ages 2, 6, 7, 11, and 15 years	Speech problems (any age) more common in those who developed schizophrenia than controls (OR=2.8)
		Milestone delays	Health visitors records completed at age 2 years	Schizophrenia cases were less likely to have learnt to talk or sit, stand, or walk unaided than controls (OR=4.8)
Bearden et al. (2000)	National Collaborative Perinatal Project (NCCP: N=2,085)	Abnormal speech	Examination by a speech pathologist at age 7 years	Abnormal speech rating significantly predicted schizophrenia (OR=12.7)
		Poor expressive language	Auditory-vocal association test at age 7 years	Individuals who later developed schizophrenia showed poorer expressive language skills than controls (OR=0.7)
Rosso et al. (2000)	NCCP (N=6,473)	Motor coordination problems	Assessment of motor skills at age 7 years	Schizophrenia cases more likely to have motor coordination problems than controls (OR=2.4)
Isohanni et al. (2001)	Northern Finland 1966 Birth Cohort (N=9,901)	Motor milestone delays	Caregiver report and examination by a research clinician at age 1 year	Age at which child learned to stand and walk unaided were both significantly associated with schizophrenia at follow-up (risk increased with later age)
Cannon et al. (2002)	Dunedin Multidisciplinary Health and Development Study (MHDS: N=976)	Language development	Standardised language tests at ages 3, 5, 7, and 9 years	Those who later developed schizophreniform disorder showed poorer receptive language skills than controls
		Motor milestone delays	Maternal self-report at age 3 years	Schizophreniform disorder cases did not start to walk until significantly later in childhood than controls

Note. N: number of participants contributing to specific analysis; controls: individuals without schizophrenia/psychotic disorder; OR: odds ratio.

Table 3. (continued)

Study	Sample	Risk factor	Method	Relationship to schizophrenia outcome
Sørensen et al. (2010)	Copenhagen Perinatal Cohort (N=5,765)	Range of developmental milestones	Maternal self-report (structured diary) during first year of life	Schizophrenia cases were later to attain developmental milestones (head lifting, sitting unsupported, crawling, and walking unsupported) than controls
Welham et al. (2010)	Mater-University Study of Pregnancy (MUSP: N=3,801)	Receptive language Ability	Standardised vocabulary test at age 5 years	In males, but not females, individuals who developed non-affective psychosis had poorer vocabulary at age 5 than controls
McNeil et al. (2011)	Swedish High-risk Project (N=166)	Motor development	Motor milestones assessed at multiple time-points	Delayed motor milestone attainment associated with risk of schizophrenia-spectrum disorder (OR=4.5)
Social, emotional, and behavioural problems				
Jones et al. (1994)	British 1946 Birth Cohort (N=4,746)	Social anxiety and withdrawal	Child self-report personality inventory at age 13 years	Linear association between social anxiety and risk for schizophrenia
			Teacher ratings at age 15 years	Schizophrenia cases appeared more anxious in school than controls
Amminger et al. (1999)	New York High-Risk Project (N=185)	Behavioural problems	Caregiver interview at age 9 years	Individuals who developed schizophrenia had higher levels of behavioural problems than controls
Crow et al. (1995)	National Child Development Study (N=12,537)	Social maladjustment	Teacher ratings of social adjustment at age 7 and 11 years	Schizophrenia cases characterised as more anxious and hostile at age 7 (males) and more withdrawn and depressed at age 11 (females) than controls

Note. N: number of participants contributing to specific analysis; controls: individuals without schizophrenia/psychotic disorder; OR: odds ratio.

Table 3. (continued)

Study	Sample	Risk factor	Method	Relationship to schizophrenia outcome
Bearden et al. (2000)	NCCP (N=8,356)	Social maladjustment	Clinician assessment at age 8 months, and 4 and 7 years	Social maladjustment at age 7 years more common among schizophrenia cases than controls (OR=2.5)
		Behavioural deviance	Clinician assessment at ages 4 and 7 years	Schizophrenia cases more likely to show deviant behaviour at age 4 (OR=1.7) and 7 (OR=1.7) than controls
Cannon et al. (2002)	Dunedin MHDS (N=976)	Internalising problems	Rutter Internalising Scales [1] at 5, 7, 9, and 11 years	Schizophreniform disorder cases obtained higher ratings on internalising scale at age 5-11 years than controls
		Externalising problems	Rutter Externalising Scales [1] at 5, 7, 9, and 11 years	Schizophreniform disorder cases obtained higher ratings on externalising scale at age 5-11 years than controls
Carter et al. (2002)	Copenhagen High-Risk Project (N=311)	Behavioural problems	Teacher rated classroom behaviours at age 15 years	Cases with schizophrenia had higher scores on disruptive behaviour scale than controls
Kim-Cohen et al. (2003)	Dunedin MHDS (N=976)	Internalising disorders	Psychiatric diagnoses at ages 11 and 15 years	Anxiety (OR=2.5) and depression (OR=7.4) more common in schizophreniform disorder cases than controls
		Externalising disorders	Psychiatric diagnoses at ages 11 and 15 years	Schizophreniform disorder cases more likely to have had ODD/CD (OR=2.8) and ADHD (OR=4.5) than controls
Schiffman et al. (2004)	Danish High-Risk Cohort (N=265)	Social deficits	Ratings of video recordings obtained at age 11-13 years	Schizophrenia cases had lower scores on a sociability scale than controls
Welham et al. (2009b)	MUSP (N=3,573)	Externalising problems	Maternal reports on CBCL [2] at ages 5 and 14 years	Non-affective psychosis predicted by high aggression scale scores at both 5 and 14 years in males (OR=6.8)

Note. N: number of participants contributing to specific analysis; controls: individuals without schizophrenia/psychotic disorder; OR: odds ratio. [1] Rutter Child Scales (Rutter et al., 1970) completed by parent and teacher; [2] Child Behaviour Checklist (Achenbach, 1991).

Table 3. (continued)

Study	Sample	Risk factor	Method	Relationship to schizophrenia outcome
Psychotic-like experiences (PLEs)				
Poulton et al. (2000)	Dunedin MHDS (N=761)	Psychotic-like experiences	Clinical interview (DISC [3]) completed at age 11 years	Schizophreniform disorder cases more likely to report 'strong' PLEs (OR=16.4) than controls
Welham et al. (2009b)	MUSP (N=3,573)	Psychotic-like experiences	YSR [4] Thought problems subscale age 14 years	Non-affective psychosis associated with auditory hallucinations in males (OR=5.1) and females (OR=2.3)
Fisher et al. (2013a)	Dunedin MHDS (N=789)	Psychotic-like experiences	Clinical interview (DISC [3]) completed at age 11 years	Children reporting PLEs at greater risk of developing schizophrenia (RR=7.2) than children who did not
Zammit et al. (2013)	Avon Longitudinal Study of Parents and Children (N=4,724)	Psychotic-like experiences	Psychosis-Like Symptom Interview at age 12 years	Psychotic disorder at age 18 associated with definite PLEs at age 12 (OR=12.7)

Note. N: number of participants contributing to specific analysis; controls: individuals without schizophrenia/psychotic disorder; OR: odds ratio; RR: risk ratio.
[3] Diagnostic Interview Schedule for Children (Costello et al., 1982); [4] Youth Self-Report (Achenbach, 1991).

1.4.2 Community-based screening approach

Screening questionnaires

A novel community-based screening method was developed to identify children presenting the triad of antecedents. Chapter 3 provides full details of the procedure and the measures included in the screening questionnaire. In brief, questionnaires including items assessing the triad of antecedents were completed by children aged 9-12 years at school and by their caregivers at home. Delays or abnormalities in speech and/or motor development were assessed via caregiver-report using quantitative and qualitative questions, which demarcated gross deviations in milestone attainment and professional and parental concerns regarding speech or motor development. Social, emotional, and behavioural problems were defined as a score in the clinical range (approximating the top tenth percentile on UK population norms) on at least one of the four Strengths and Difficulties Questionnaire [SDQ (Goodman, 2001)] psychopathology scales, including, child-reported emotional symptoms, or caregiver-reported conduct problems, hyperactivity-inattention, or peer relationship problems. The presence of a psychotic-like experience was defined as at least one child-reported 'certainly-true' response on a nine-item PLE measure (Laurens et al., 2007; Laurens et al., 2011).

Prevalence of the antecedent triad

An initial pilot study examining data from 264 children and their primary caregivers demonstrated that the community screening approach provided a cost-effective and feasible method of identifying children presenting the triad of antecedents (Laurens et al., 2007). A subsequent publication reported school-based questionnaire screening data from 1,347 children in primary schools across Greater London (Laurens et al., 2011). Of these, 26% were reported to have experienced any speech and/or motor delay or abnormality, 32% presented with abnormal scores on at least

one of the four SDQ psychopathology scales (emotional symptoms: 12%; conduct problems: 11%; hyperactivity-inattention: 10%; peer relationship problems: 14%), and 63% reported at least one certainly-true PLE (Laurens et al., 2011). The percentage of children reporting each individual PLE ranged from 9% to 34%; the most commonly reported experiences being auditory hallucinations (34%), paranoid thoughts (30%), and visual hallucinations (27%). In total, 10% of children met ASz criteria and 24% presented with none of the antecedents. The majority of ASz children (69%) reported that the PLEs they experienced were either distressing or associated with impairment.

Demographic correlates

In accordance with the finding that males are at greater risk for schizophrenia (Tandon et al., 2009), the ASz triad was more common among boys than girls (13% vs. 6%) (Laurens et al., 2011). Relative to white British children, those of African-Caribbean, black African, and 'other' ethnicity were significantly more likely to present the ASz triad (Laurens et al., 2008; Laurens et al., 2011); although migrant status was not associated with any of the antecedent components. The higher prevalence of the ASz triad among African-Caribbean and black African children is consistent with the elevated rates of psychosis observed among these groups in the UK (Kirkbride et al., 2012), which may reflect differences in the distribution of socio-environmental risk factors across ethnic groups.

1.4.3 Characteristics of ASz children

Whilst only longitudinal follow-up can determine the specificity and sensitivity of the triad in predicting later schizophrenia, preliminary investigations comparing ASz children to their typically-developing (TD) peers (who do not present with any of the antecedents or a family history of illness) indicate that ASz children display several features that characterise adults with established illness.

Neurocognitive function

Impairments in neurocognitive function, equating to a moderate effect size (*d*=0.52), have been observed among ASz children aged 9-12 years (Cullen et al., 2010). In this study, ASz children obtained lower scores than the TD group on all 16 neurocognitive subtests examined. Significant group differences were observed for four domains: general intelligence, verbal memory, working memory, and executive function – inhibition (Figure 2), with the largest impairments observed in working memory. These neurocognitive impairments are less pronounced than those reported in individuals with schizophrenia (Mesholam-Gately et al., 2009), but are of a similar magnitude to those observed in young adult relatives (Agnew-Blais & Seidman, 2013), youth at UHR (Fusar-Poli et al., 2012b; Giuliano et al., 2012), individuals with SPD (Siever & Davis, 2004; Seeber & Cadenhead, 2005; Raine, 2006), and children reporting PLEs (Kim et al., 2012; Hameed et al., 2013; Kelleher et al., 2013a).

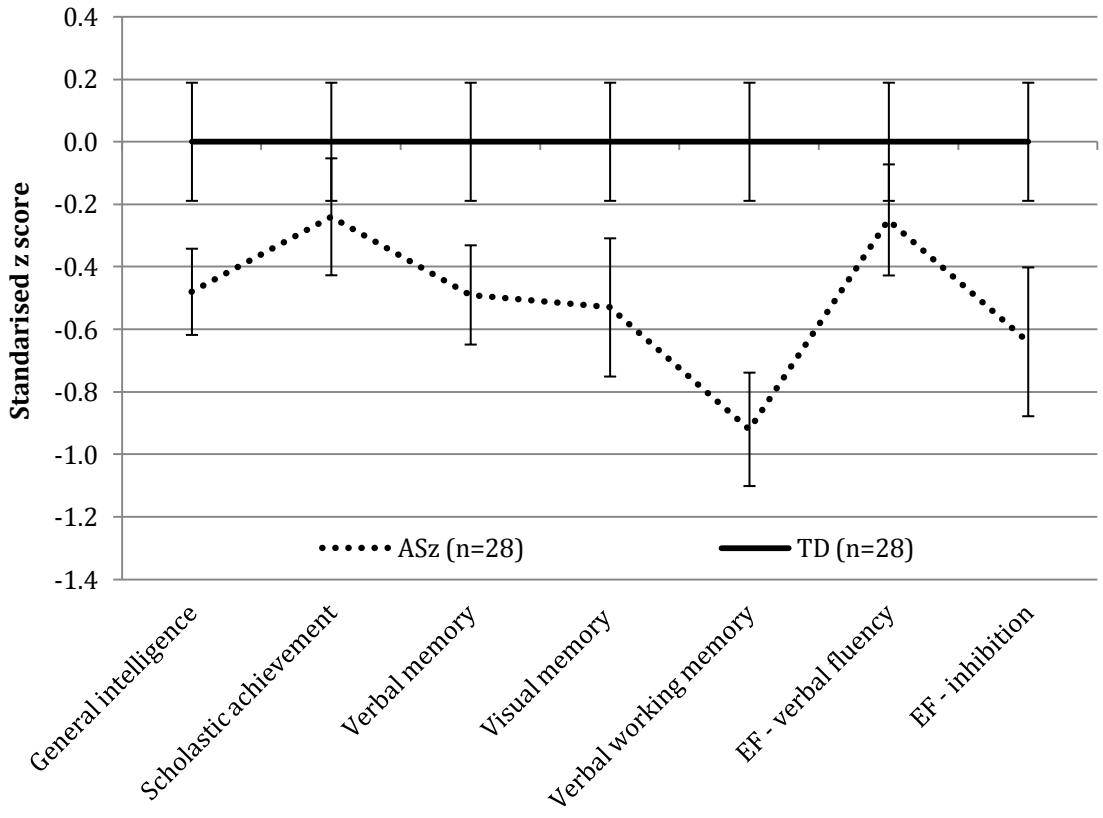

Figure 2. Neurocognitive performance in ASz children relative to TD children

Note. Figure adapted from Cullen et al. (2010). Lines indicate mean (±SE). EF: Executive function.

Event-related processing

An event-related processing (ERP) study also provided evidence that ASz children are characterised by abnormalities of brain function similar to those observed in patients with schizophrenia (Laurens et al., 2010). Twenty-two ASz and 26 TD children completed a Go/No-Go ERP paradigm indexing error-related negativity (ERN), which describes the peak in brain activity that follows the detection of an erroneous response. Like adults with schizophrenia and their unaffected relatives (Bates et al., 2004; Simmonite et al., 2012), ASz children were found to show reduced amplitude of the ERN component compared to the TD group. This reduction in amplitude is thought to reflect functional abnormality of the anterior cingulate cortex, which, as noted in preceding sections, is a region in which reduced grey matter volume has been observed among individuals with a family history of illness (Fusar-Poli et al., in press).

A subsequent ERP study examined performance on an auditory oddball task in 22 ASz and 24 TD children matched on demographic variables, pubertal status, and IQ (Bruggemann et al., 2013). This task was used to investigate mismatch negativity (MMN), an ERP component that is observed when the brain detects a change in auditory stimuli (i.e., a discrepancy between an incoming stimulus and the memory of previously presented stimuli). Relative to the TD group, ASz children were found to show *increased* MMN amplitude at the frontal sites. Interestingly, individuals with schizophrenia have been found to show a *decrease* in MMN amplitude relative to healthy controls (Turetsky et al., 2007). The pattern of abnormality that was found to characterise ASz children is therefore different to that which is typically observed among individuals with schizophrenia, and may demonstrate important stage effects in the evolution of the MMN abnormality in schizophrenia.

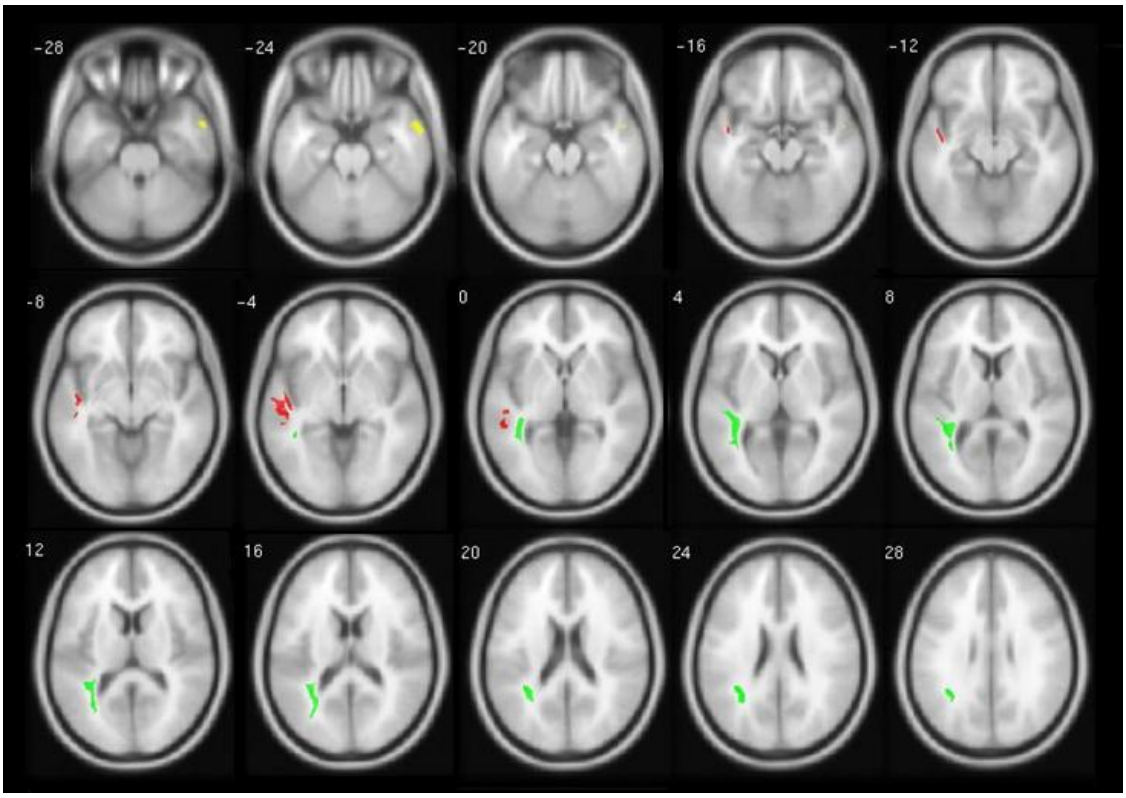

Figure 3. Significant clusters of relatively increased and decreased grey matter and white matter volume in ASz children relative to TD children

Note. Figure adapted from (Cullen et al., 2013). Relatively decreased (yellow) and increased (red) grey matter volume, as well as relatively increased white matter volume (green) in ASz children (n=20) compared to the TD group (n=20) (p<0.05, corrected for multiple comparisons).

Whole brain structure

Voxel-based morphometry techniques have demonstrated that ASz children show differences in grey matter (GM) and white matter (WM) volume compared to TD children (Cullen et al., 2013). GM volume was significantly decreased in the right middle temporal gyrus in ASz children and significantly increased in the left superior-middle temporal gyri (Figure 3). Additionally, ASz children showed increased WM volume in a cluster encompassing the left inferior parietal lobe, occipital lobe, and superior temporal gyrus. These structural brain abnormalities comprise a subset of the regions affected in first-episode psychosis patients and UHR youth (Fusar-Poli et al., 2012c; Shepherd et al., 2012). A similar pattern of abnormalities was also observed in study of children reporting PLEs at interview (Jacobson et al., 2010).

Involuntary dyskinetic movements

ASz children have also been found to show higher levels of involuntary dyskinetic movements compared to their TD peers (Macmanus et al., 2012), thought to be an indication of abnormal striatal dopamine regulation. Using an established procedure (Walker et al., 1994), blind ratings of videotapes were used to assess involuntary dyskinetic movements in 21 ASz and 31 TD children. ASz children were found to present with significantly more movement abnormalities in the facial regions (e.g., tics, grimacing, blinking, tongue thrusts) and upper body (e.g., shoulder/hip torsion, writhing and extensions of the fingers or wrist). These abnormalities are similar to those that have been observed among children who later develop schizophrenia (Walker et al., 1994; Rosso et al., 2000) and adolescents with SPD (Mittal et al., 2007).

Facial emotional processing

ASz children have also been found to show moderate impairments in their ability to recognise facial emotions compared to TD children (Dickson et al., in press-a), with the largest impairments found for sad and angry facial emotions. Additionally, relative to the TD group (n=34), ASz children (n=34) more commonly misattributed neutral expressions to other facial emotions and mislabelled neutral emotions as sad. These impairments are similar to those observed in individuals with schizophrenia (Kohler et al., 2010) as well as those found in UHR youth (Thompson et al., 2011b) and first-degree relatives of individuals with schizophrenia (Lavoie et al., 2013).

Social withdrawal

Finally, a study of 38 ASz children, 49 TD children, and 34 children with a family history of schizophrenia (Matheson et al., 2013b) investigated social withdrawal using parent-reported scores on the Child Behaviour Checklist (Achenbach & Rescorla, 2001). ASz children were characterised by higher scores on the social withdrawal scale relative to their typically-developing peers, equating to a moderate-

to-large effect size (*d*=0.74). Children with a family history of schizophrenia were also found to present with higher levels of social withdrawal relative to TD children; however, the effect size observed in the family history group (*d*=0.44) was smaller in magnitude than that observed among ASz children. These findings are consistent with studies showing that social withdrawal characterises children who go on to develop schizophrenia in later life (Tarbox & Pogue-Geile, 2008).

1.5 Summary

Strategies to identify individuals at elevated risk for schizophrenia have focused on individuals with a family history of illness, UHR youth, individuals with SPD, and those reporting PLEs. Evidence suggests that these groups are characterised by many of the neurobiological abnormalities and social cognitive impairments observed among individuals with established illness, although these abnormalities are typically smaller in magnitude. Such studies have enabled the characterisation of the premorbid stages of schizophrenia which may ultimately lead to more effective interventions being delivered during the early stages of psychosis to reduce the risk of illness progression. Limitations associated with established strategies for identifying high-risk individuals have motivated the development of a novel approach which focuses on children aged 9-12 years who present multiple antecedents of schizophrenia. Research has demonstrated that these children can be identified using a cost-effective school-screening procedure. Whilst only longitudinal follow-up will confirm the extent to which ASz children are at elevated risk for schizophrenia, preliminary investigations show that these children share several characteristics with adults who have the disorder and other high-risk groups.

CHAPTER 2 Systematic review of stress and HPA axis function in individuals at elevated risk for schizophrenia

2.1 Introduction

As proposed by the neural diathesis-stress model of schizophrenia (Walker & Diforio, 1997; Walker et al., 2008), psychosocial stress exposure may elicit abnormality within the HPA axis that contributes to the onset of psychosis among individuals with an underlying vulnerability for the disorder. Several lines of evidence support this model. Firstly, there is evidence that exposure to psychosocial stressors (e.g., major life events, childhood trauma, and milder daily hassles) contributes to the development and maintenance of psychosis (Myin-Germeys & van Os, 2007; Phillips et al., 2007; Varese et al., 2012) and that individuals with psychosis show greater emotional reactivity to potentially stressful experiences than healthy individuals (Myin-Germeys et al., 2001). Secondly, abnormal HPA axis function, as indexed by elevated diurnal cortisol levels and/or a blunted cortisol awakening response (CAR), has been observed in individuals with first-episode psychosis (Borges et al., 2013) and has also been associated with neurocognitive deficits in this population (Aas et al., 2011b). Finally, patients with first-episode psychosis have also been found to show enlarged pituitary volumes (Borges et al., 2013; Nordholm et al., 2013). Whilst these studies provide evidence that psychosis is characterised by increased exposure and reactivity to psychosocial stressors and HPA axis dysfunction, the extent to which these features precede illness onset is currently unclear.

Two recent systematic reviews have investigated whether individuals at elevated risk for schizophrenia are also characterised by increased stress susceptibility and HPA axis dysfunction (Aiello et al., 2012; Nordholm et al., 2013). The first reported that individuals with a family history of illness and UHR youth are characterised by

increased emotional reactivity to stress and enlarged pituitary volume relative to controls (Aiello et al., 2012), but that only UHR youth showed elevated cortisol levels. This review did not compute standardised effect sizes to allow comparisons across high-risk groups and did not include at-risk individuals with SPD or those reporting PLEs. Moreover, studies examining psychosocial stress exposure (as opposed to stress reactivity) were not included. The second review, which focused specifically on pituitary volume abnormalities, reported that UHR youth and individuals with SPD were characterised by increased pituitary volume relative to controls (Nordholm et al., 2013); standardised effect sizes demonstrated that the observed differences were small in magnitude. This review did not include studies examining individuals with a family history of illness or those reporting PLEs. Thus, it is currently unclear as to whether all high-risk groups are characterised by increased exposure and reactivity to psychosocial stressors and HPA axis abnormalities, and whether effect sizes are similar across high-risk groups. The following chapter comprises a systematic review of studies examining experiences of psychosocial stress, cortisol levels, and pituitary volume among relatives of patients with schizophrenia, UHR youth, individuals with SPD, and those reporting PLEs. The specific aims were as follows:

1. Review studies examining (i) exposure and reactivity to psychosocial stressors, (ii) cortisol levels, and (iii) pituitary volume in order to determine the magnitude of differences between high-risk and healthy individuals.

2. Evaluate the extent to which experiences of psychosocial stress (exposure and reactivity) and HPA axis abnormalities in high-risk individuals are associated with psychopathology and illness progression.

3. Discuss methodological considerations pertinent to the work presented in Chapters 4, 5, 6, and 7 and describe how this thesis will address these issues.

2.2 Methods

2.2.1 Data sources and search terms

Systematic literature searches were conducted within PubMed, PsycINFO, and EMBASE during September 2013. The following search terms were employed: "stress; hassle; life event; trauma; stressor; abuse; maltreatment; adversity; victimisation; neglect; cortisol; or pituitary" in combination with "relatives; offspring; sibling; family history; genetic risk; at risk mental state; ultra high risk; clinical high risk; prodrome; or high risk" and "schizophrenia or psychosis" or "schizotypal personality disorder; schizotypy; psychotic experiences; psychotic-like experiences; subclinical psychotic symptoms; subclinical psychosis; or non-clinical psychosis". Searches were conducted in two waves; the first wave included psychosocial stress terms in combination with the high-risk search terms, and the second wave included cortisol and pituitary in combination with high-risk key words. Reference lists of review articles were manually searched to identify additional studies.

2.2.2 Inclusion criteria

The search was restricted to published studies only; conference abstracts and dissertations were excluded, as were studies not written in English. As the aim of the review was to determine whether individuals at elevated risk for psychosis are (i) more likely to encounter psychosocial stressors, (ii) show increased reactivity to psychosocial stressors, and (iii) are characterised by biological markers of HPA axis dysfunction relative to healthy controls, prospective studies examining the extent to which these factors precede the satisfaction of at-risk criteria (i.e., where the at-risk status may have evolved as a consequence of these features) were excluded. Similarly, retrospective studies examining the relationship between risk status and prior exposure to psychosocial stressors were not included if there was a clear passage of time between exposure occurrence and the onset of at-risk symptoms

(e.g., studies examining childhood trauma in adult populations). Association studies that used multivariate models to predict PLEs (i.e., where the univariate relationship between PLEs and experiences of psychosocial stress/HPA axis dysfunction could not be ascertained) were also not eligible for inclusion.

The review therefore included cross-sectional studies examining the association between high-risk status and experiences of psychosocial stress (exposure and/or reactivity), cortisol levels, or pituitary volume, or prospective studies of high-risk individuals which assessed these outcomes at follow-up. The review included studies examining cortisol responses elicited by psychosocial stressor tasks (i.e., experimental tasks designed to induce an increase in cortisol levels). As it was beyond the scope of this review to examine cortisol secretion following pharmacological challenge, studies measuring responses to metabolic stressors (e.g., dexamethasone) were only included if baseline or placebo condition data were reported, thereby providing a measure of basal cortisol levels. Studies that did not include a healthy control group were not examined in the systematic review; however, those that assessed the extent to which experiences of psychosocial stress or HPA axis function were associated with psychopathology or illness progression were described in sections focusing on these outcomes. When two studies were found to examine overlapping samples, the first (i.e., original) study was included in the systematic review. Study inclusion was determined by the candidate. Titles and abstracts were first reviewed to identify potentially relevant studies; full texts of these studies were then examined to determine those that met full inclusion criteria.

2.2.3 Data extraction and analyses

Search results were exported into an Endnote database in order to identify duplicate studies. The database was subsequently transferred to a Microsoft Excel spreadsheet where manual cleaning was performed to remove additional duplicates and studies

were examined for eligibility. The following data were then extracted from studies that met inclusion criteria: (i) sample characteristics (sample size of high-risk and healthy control groups, and mean ages for each group), (ii) outcome measures (assessment of exposure and/or reactivity to psychosocial stressors, cortisol sampling protocol, or pituitary volume analysis software), and (iii) data necessary to compute standardised mean differences (*d*) in psychosocial stressor exposure, psychosocial stress reactivity, cortisol levels, or pituitary volume among high-risk individuals relative to healthy controls. Standardised mean differences were computed from means and standard deviations or derived from odds ratios using established methods (Lipsey & Wilson, 2001). Effect sizes of 0.20, 0.50, and 0.80 corresponded to 'small', 'medium', and 'large' effects, respectively (Cohen, 1992). The diversity of measures employed across studies prevented the ability to perform a meta-analysis of these effects.

2.3 Search results

Figure 4 summarises the search results and the reasons for study exclusion. Database searches yielded 7114 published studies in total. Two further studies were identified through manual searching. After removing duplicates, 6099 unique studies were identified. Of these, 5911 studies were excluded after reviewing the abstract; a further 150 were excluded after the full text was reviewed. The systematic review examining differences between high-risk individuals and healthy controls included 38 studies in total (Schuldberg et al., 1996; Neumann & Walker, 1999; Weinstein et al., 1999; Miller et al., 2001; Myin-Germeys et al., 2001; Walker et al., 2001; Marcelis et al., 2004; Mitropoulou et al., 2004; Garner et al., 2005; De Loore et al., 2007; Mittal et al., 2007; Spelman et al., 2007; Brunelin et al., 2008; Kelleher et al., 2008; Mondelli et al., 2008; Takahashi et al., 2009; Zong et al., 2010; Büschlen et al., 2011; Collip et al., 2011; Pruessner et al., 2011; Soliman et al., 2011; Tessner et al., 2011; Yıldırım et al., 2011; Habets et al., 2012; Mizrahi et al., 2012; Palmier-Claus et al., 2012; Phillips et al., 2012; Sugranyes et al., 2012; Yang et al., 2012; Addington et al., 2013; Devylder et al., 2013; Kelleher et al., 2013c; Mittal et al., 2013; Pruessner et al., 2013a; Romo-Nava et al., 2013; Sahin et al., 2013; Tikka et al., 2013; Walker et al., 2013). Of these, 15 examined exposure or reactivity to psychosocial stressors, 17 examined basal cortisol levels or cortisol responses during psychosocial stressor tasks, and 6 examined pituitary volume. These studies included data from 2432 high-risk individuals and 3742 healthy controls. Nine additional studies (Mason et al., 2004; Myin-Germeys et al., 2005a; Thompson et al., 2007; Thompson et al., 2009; Bechdolf et al., 2010; Walker et al., 2010; Takahashi et al., 2011; Corcoran et al., 2012; Thompson et al., in press) which did not meet inclusion criteria but which examined the extent to which psychosocial stress or HPA axis function was associated with psychopathology or symptom progression are also discussed.

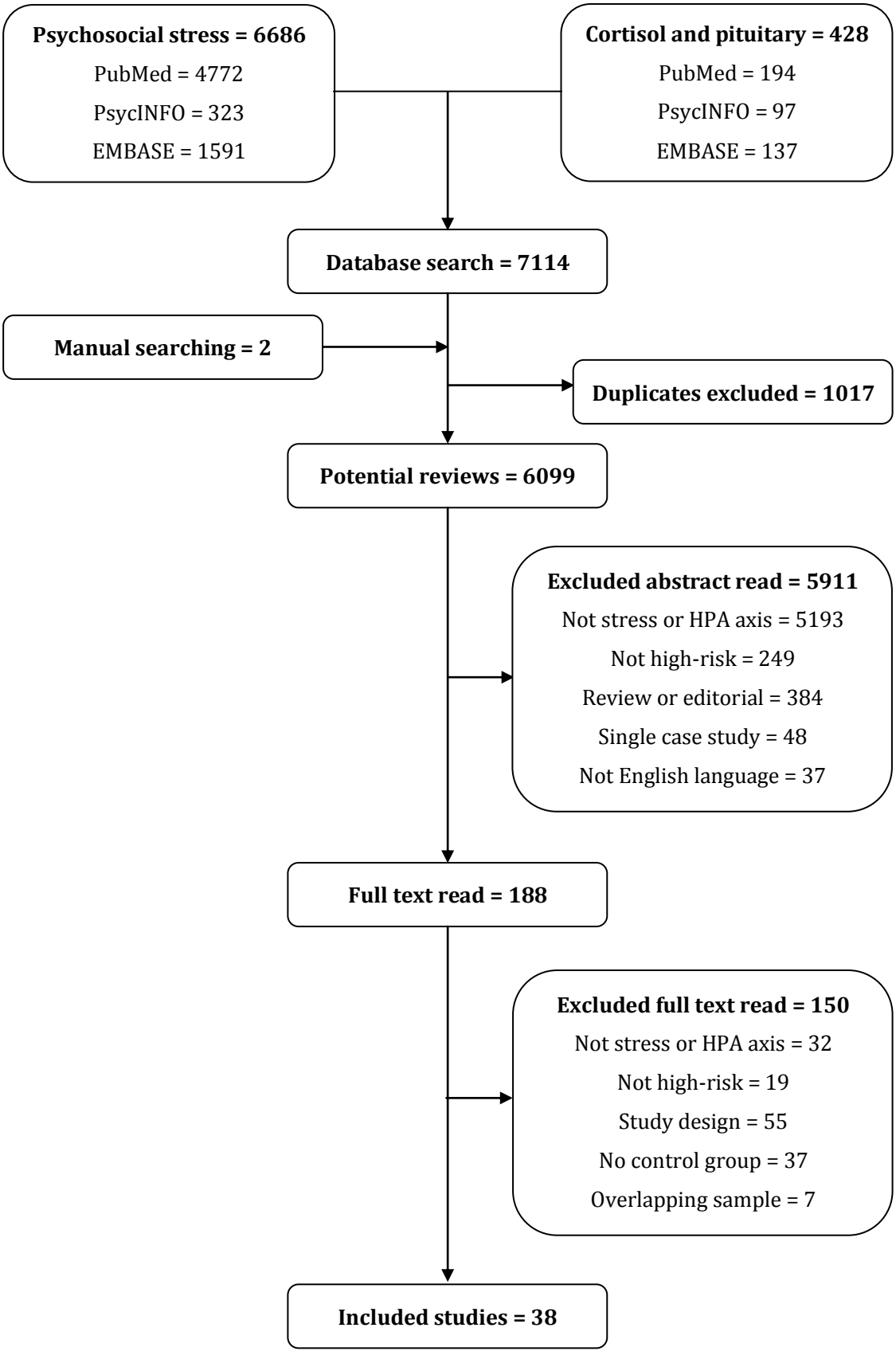

Figure 4. Systematic review search strategy

2.4 Psychosocial stress

2.4.1 Psychosocial stress in high-risk individuals compared to healthy controls

Studies examining experiences of psychosocial stress among high-risk individuals and healthy controls are presented in Table 4 (ordered chronologically), with effect sizes (*d*) computed to indicate the magnitude of group differences.

Individuals with a family history of illness

Only two studies of individuals with a family history of psychosis met inclusion criteria. Miller and colleagues examined exposure to life events in a sample of high-risk youth (age range: 16-25 years) who were selected on the basis of having at least two first- or second-degree relatives with schizophrenia (Miller et al., 2001). In this study, no difference was observed in the number of major or minor life events experienced by high-risk youth relative to healthy controls. The second study (Myin-Germeys et al., 2001) assessed emotional reactivity to psychosocial stress using the Experience Sampling Method (ESM), a structured diary technique which assesses exposure to stressful experiences and current mood in the context of daily life (Myin-Germeys & van Os, 2007). Participants are prompted by a digital wristwatch at random intervals throughout the day to describe stressful experiences (event-related, activity-related, thought-related, and social stress) and provide ratings of current mood and symptoms. The study of adult first-degree relatives of individuals with psychosis (age range: 18-55 years) employing the ESM technique found no differences in the number of stressful experiences reported by relatives and healthy controls (Myin-Germeys et al., 2001). However, relatives showed greater decreases in positive affect in response to stressful experiences compared to healthy controls, and, to a lesser extent, greater increases in negative affect. Changes in positive and negative affect among relatives compared to controls were moderate-to-large and small-to-moderate in magnitude, respectively.

Youth at ultra high risk for psychosis

Seven studies were identified which examined experiences of psychosocial stress among UHR youth (age range: 12-35 years). Three studies assessed exposure to trauma prior to age 18 years (abuse, neglect, and bullying), but were included as the age range of participants overlapped with the exposure period (Addington et al., 2013; Sahin et al., 2013; Tikka et al., 2013). All three studies reported that UHR youth were characterised by higher levels of trauma than healthy controls, with moderate-to-large effect sizes. In contrast, two studies reported that UHR youth experienced fewer recent life events than healthy controls (Phillips et al., 2012; Devylder et al., 2013). Group differences were larger and only statistically significant in the former study (Phillips et al., 2012); however, UHR youth in this study were more distressed by these events than healthy controls. Similarly, this study also observed no differences in the number of recent hassles experienced by UHR youth and healthy controls (Phillips et al., 2012), yet UHR youth rated the hassles they experienced as significantly more intense. The ESM technique has also been employed in a recent study of UHR youth (Palmier-Claus et al., 2012). Relative to healthy controls, the UHR group experienced significantly greater social stress, equating to a large effect size, and showed a greater increase in negative affect than controls following exposure to each type of stressor. However, effect sizes could not be computed for negative affect; thus, it is not clear whether the magnitude of difference is equivalent to that observed in the study of individuals with a family history of psychosis (described in the previous section). Youth at UHR have also been reported to experience higher levels of perceived stress than healthy controls (Palmier-Claus et al., 2012; Phillips et al., 2012) and greater levels of impaired stress tolerance (Devylder et al., 2013), with large effect sizes reported for both measures. However, another study found that UHR youth and healthy controls did not differ on levels of chronic stress related to work and social relationships (Pruessner et al., 2011).

Individuals with schizotypal personality disorder

Two studies were identified that assessed psychosocial stress in university/college students (age range: 18-25 years) characterised by high scores on measures of schizotypal traits. The first reported that among youth with schizotypal features, those with anhedonic traits, but not those with perceptual aberrations and magical ideas, experienced a higher frequency of daily hassles than healthy controls (Schuldberg et al., 1996), equating to a large effect size. However, youth with anhedonic traits rated the hassles they experienced as being less intense than those experienced by healthy controls. Furthermore, although both groups differed from the control group on their ability to cope with stressful events, no consistent pattern emerged (i.e., youth with schizotypal traits showed both poorer and better coping abilities). The more recent of these studies found that relative to healthy controls, youth with schizotypal traits experienced a higher number of stressful life events and were more distressed by these experiences (Zong et al., 2010), with moderate-to-large effect sizes observed for both outcomes. Only one study examining youth with a diagnosis of SPD met criteria for inclusion. Adolescents with SPD (age range: 12-18 years) experienced a significantly greater number of life events during the past year than their healthy peers, with a larger magnitude of differences observed for undesirable life events (Tessner et al., 2011). Furthermore, although youth with SPD did not report a higher number of daily hassles than healthy controls, they were more distressed by these experiences, with moderate-to-large differences observed.

Two studies using data from a large, nationally-representative sample in the United States (Afifi et al., 2011; Afifi et al., 2012) did not meet criteria for inclusion in the systematic review, but provide further evidence that SPD is associated with psychosocial stress. The first of these studies (Afifi et al., 2011) reported that SPD was significantly associated with all types of childhood trauma (physical, sexual, and

emotional abuse, and neglect). Subsequently, it was found that SPD was associated with physical punishment (i.e., being pushed, grabbed, shoved, slapped, or hit by parents or other caregivers), even in the absence of childhood maltreatment (Afifi et al., 2012).

Youth reporting psychotic-like experiences

Three studies of individuals reporting PLEs were found to meet inclusion criteria; all three examined the association between PLEs and traumatic experiences in childhood. A cross-sectional study found that children reporting PLEs (age range: 12-15 years) were more likely to have experienced physical abuse and to have witnessed domestic violence than their healthy peers (Kelleher et al., 2008), both exposures were associated with large effect sizes. Additionally, two large prospective studies were identified that examined the extent to which adolescents reporting PLEs (age range: 13-16 years) were at greater risk of experiencing trauma at follow-up, after controlling for trauma exposure at baseline. De Loore and colleagues found that adolescents who reported PLEs at baseline were more likely to experience negative life events during a two-year follow-up compared to adolescents who had not reported PLEs (De Loore et al., 2007); however, adolescents reporting PLEs were not at significantly greater risk of unwanted sexual experiences or bullying. It was not possible to compute effect sizes for these exposures. More recently, it was observed that adolescents reporting PLEs at baseline were at significantly greater risk of experiencing physical assault and bullying during a three-month follow-up than adolescents who did not report PLEs (Kelleher et al., 2013c), with both outcomes associated with large effect sizes. Additionally, PLEs remained significantly associated with physical assault during a 12-month follow-up, but the association with bullying reduced to trend level.

Table 4. Studies examining psychosocial stress in high-risk individuals relative to healthy controls

Study	Sample	Age (mean)	Measure	Findings	*d*
Individuals with a family history of psychosis (FHx)					
Miller et al. (2001)	FHx (*n*=155) HC (*n*=36)	FHx=21.0 HC=NR	Schedule of Recent Experiences [1] (number of life events ever)	No sig difference in the number of life events in FHx vs. HC	Insufficient data to compute
Myin-Germeys et al. (2001)	FHx (*n*=47) HC (*n*=49)	FHx=36.5 HC=35.2	ESM: event-, activity-, thought-, and social-related stressful experiences and emotional reactivity (PA and NA)	No sig difference in stress exposure; FHx greater decreases in PA and increases in NA than HC	Stress (range): -0.17 – 0.53 PA (range): -0.73 – -1.31 NA (range): 0.29 – 0.58
Youth at ultra high-risk (UHR)					
Pruessner et al. (2011)	UHR (*n*=30) HC (*n*=30)	UHR=20.3 HC=22.5	Trier Inventory for the Assessment of Chronic Stress [2] (chronic stress related to work and relationships)	No sig difference in chronic stress between UHR and HC	Insufficient data to compute
Palmier-Claus et al. (2012)	UHR (*n*=27) HC (*n*=27)	UHR=22.6 HC=22.6	ESM (described above); Perceived Stress Scale [3]	UHR sig higher ESM social stress, greater increases in NA, and higher levels of perceived stress than HC	Social stress: 0.83 Perceived stress scale: 1.23
Phillips et al. (2012)	UHR (*n*=143) HC (*n*=32)	UHR=18.7 HC=21.5	Life Events Interview Schedule [4] (past month); Kanner hassles scale [5] (past month); Perceived Stress Scale [3]	UHR sig fewer life events but greater life event distress, greater intensity of hassles, and higher levels of perceived stress than HC	Life event exposure: -0.74 * Life event distress: 0.77 Hassles intensity: 0.83 Perceived stress scale: 1.13

Note. HC: Healthy controls; NR: not reported; sig: significant; ESM: Experience Sampling Method; PA: positive affect; NA: negative affect; *d*: standardised effect size derived from means. * Computed from age-adjusted means. [1] (Paykel et al., 1971); [2] (Schulz & Schlotz, 1999); [3] (Cohen et al., 1983); [4] (Ventura et al., unpublished); [5] (Kanner et al., 1981).

Table 4. (continued)

Study	Sample	Age (mean)	Measure	Findings	*d*
Addington et al. (2013)	UHR (*n*=360) HC (*n*=180)	UHR=19.0 HC=19.5	Childhood Trauma and Abuse Scale [6] (physical abuse, psychological abuse, sexual abuse, and neglect)	UHR sig more likely to experience all types of abuse and neglect than HC	Range: 0.43 – 0.69
DeVylder et al. (2013)	UHR (*n*=65) HC (*n*=24)	UHR=19.5 HC=20.4	Coddington's Life Events Record [7] (past 3 months); SOPS 'impaired stress tolerance' item	No sig difference in the number of life events; UHR sig higher scores than HC on impaired stress tolerance item	Life events: -0.29 Impaired stress tolerance item: 1.48
Sahin et al. (2013)	UHR (*n*=41) HC (*n*=69)	UHR=20.5 HC=23.9	Childhood Trauma Questionnaire [8] (physical abuse, psychological abuse, sexual abuse, and neglect)	UHR sig higher scores than HC on all trauma scales except sexual abuse	Total score: 0.74
Tikka et al. (2013)	UHR (*n*=20) HC (*n*=30)	UHR=22.2 HC=23.6	Trauma and Distress Scale [9] (sexual, physical, and emotional abuse and physical and emotional neglect)	UHR sig higher total trauma scores than HC and sig higher scores on each trauma scale	Total score: 1.54
Adolescents with schizotypal personality disorder/schizotypal traits (SPD/ST)					
Schuldberg et al. (1996)	ST-p (*n*=88) ST-a (*n*=21) HC (*n*=89)	ST=18.8 HC=19.1	Kanner hassles scale [5] (past month); Stress Questionnaire [10] (appraisal, coping, and emotions relating to events during past fortnight)	ST-a sig greater number of hassles than HC but lower intensity. ST-p and ST-a groups showed some differences to HC on coping strategies	ST-a vs. HC Hassles frequency: 0.86 Hassles intensity: -0.48

Note. HC: Healthy controls; sig: significant; SOPS: Scale of Prodromal Symptoms (Miller et al., 2003); ST-p: schizotypal traits with perceptual aberrations and magical ideas; ST-a: schizotypal traits with anhedonia; *d*: standardised effect size derived from means. [6] (Janssen et al., 2004); [7] (Coddington, 1972); [8] (Bernstein & Fink, 1998); [9] (Patterson et al., 2002); [10] (Folkman & Lazarus, 1988).

Table 4. (continued)

Study	Sample	Age (mean)	Measure	Findings	*d*
Zong et al. (2010)	ST (*n*=48) HC (*n*=48)	ST=18.4 HC=18.4	Coping Flexibility Questionnaire [11] (life event exposure and distress)	ST sig higher number of life events than HC and greater distress	Total No. life events: 0.98 Life event distress: 1.01
Tessner et al. (2011)	SPD (*n*=36) HC (*n*=52)	SPD=14.2 HC=14.1	Psychiatric Epidemiological Research Interview [12] (life events during past year) and Daily Stress Inventory [13] (hassles current day)	SPD sig higher number of total (and undesirable) life events than HC; no difference in number of hassles but greater distress relating to hassles	Total life events: 0.54 Undesirable events: 0.83 Hassles distress: 0.70
Children reporting psychotic-like experiences (PLEs)					
De Loore et al. (2007)	PLE (*n*=207) HC (*n*=922)	Not provided [a]	Single questions assessing bullying, sexual trauma, and exposure to negative life events	PLE group sig more likely to experience negative life events than HC but not bullying or sexual trauma	Insufficient data to compute
Kelleher et al. (2008)	PLE (*n*=14) HC (*n*=197)	Not provided [b]	K-SADS interview (physical and sexual abuse, domestic violence, and bullying)	PLE group sig more likely to experience physical abuse and witness domestic violence than HC	Physical abuse: 0.98 * Domestic violence: 1.27 *
Kelleher et al. (2013c)	PLE (*n*=77) HC (*n*=1035)	Not provided [c]	Single questions assessing physical assault and bullying	PLE group sig more likely to experience physical assault and bullying at follow-up than HC	Physical assault: 1.09 ** Bullying: 0.92 **

Note. HC: Healthy controls; sig: significant; K-SADS: Kiddie Schedule for Affective Disorders and Schizophrenia for School Aged Children: Present and Lifetime Version (Kaufman et al., 1997); *d*: standardised effect size derived from means. * Computed from odds ratios adjusted for sex and socioeconomic status; ** computed from crude odds ratios. [a] Total sample mean = 15.1 years; [b] total sample range = 12–15 years; [c] total sample range = 13–16 years. [11] (Cheng, 2001); [12] (Dohrenwend, 1998); [13] (Brantley et al., 1987).

2.4.2 Associations between psychosocial stress and current psychopathology

Several studies have investigated the relationship between psychosocial stress and current psychopathology among high-risk individuals. These studies potentially offer the opportunity to determine whether psychosocial stress is more strongly associated with psychopathology among high-risk individuals compared to controls (as might be hypothesised based on the diathesis-stress model), although some studies have only examined associations across the total sample (i.e., combining high-risk and healthy individuals). A study of youth with a family history of schizophrenia (age range: 16-25 years) observed that major life events (e.g., the death of a loved one), but not minor events (e.g., moving within the same city), predicted psychotic symptoms in the total sample of high-risk youth and healthy controls (Miller et al., 2001). Similarly, a study of adolescents with SPD, other personality disorders, and healthy youth (age range: 12-18 years), reported that prodromal symptoms were correlated with both the number of recent life events and the number of daily hassles when data were collapsed across diagnostic groups (Tessner et al., 2011).

Other studies have investigated the relationship between experiences of psychosocial stress and psychopathology in high-risk individuals only. Three such studies examined UHR youth (age range: 12-35 years). The first of these studies reported that the number of life events and daily hassles experienced during the past month were not associated with psychotic symptoms, depression, or anxiety (Thompson et al., 2007), although daily hassles were positively associated with global psychopathology. In two further studies, the degree of trauma exposure was associated with depression and anxiety symptoms (Thompson et al., 2009; Addington et al., 2013); in the former study, this was only true for white and not ethnic minority UHR youth, although trauma scores were correlated with positive symptoms across the entire UHR group. Another study of UHR youth (age range not provided) reported

that subjective feelings of chronic stress were associated with more severe positive and depressive symptoms (Pruessner et al., 2011). Finally, using the ESM technique, a study of adult first-degree relatives of patients with psychosis (age range: 18-55 years) observed that stressful events were associated with an increase in psychotic experiences (Myin-Germeys et al., 2005a) whilst a study of UHR youth (age range: 18-35 years) reported that these events were correlated with increased intensity of delusions and hallucinations (Palmier-Claus et al., 2012).

2.4.3 Longitudinal studies relating psychosocial stress to outcome

Longitudinal studies of high-risk individuals have examined the extent to which experiences of psychosocial stress are associated with symptom progression and transition to psychosis. In a study of adolescents with SPD (age range: 12-18 years), the number of daily stressors and undesirable life events experienced predicted an increase in positive prodromal symptoms across the total sample (i.e., adolescents with SPD, other personality disorders, and no personality disorders) at a one-year follow-up (Tessner et al., 2011). However, there were no significant associations when diagnostic groups were examined separately. In contrast, in a study which examined UHR youth (age range: 12-30 years) separately, the number of life events reported at baseline was not related to positive or negative prodromal symptoms during a four-year follow-up (Devylder et al., 2013), although impaired stress tolerance was strongly associated with later symptoms. Furthermore, in a study of individuals at UHR (age range: 13-28 years), exposure to stressful life events was not associated with transition to psychosis during a one-year follow-up (Mason et al., 2004). Consistent findings have been obtained in two studies examining trauma exposure in UHR youth (age range: 15-30 years); the first assessed a wide range of interpersonal (e.g., sexual or physical assault) and non-interpersonal trauma exposures (e.g., being involved in an accident or natural disaster) occurring at any

age (Bechdolf et al., 2010), whilst the second study examined exposure to maltreatment during childhood (Thompson et al., in press). Both studies reported that whilst total trauma scores were not related to outcome, sexual trauma was specifically associated with transition to psychosis at follow-up.

2.4.4 Summary of psychosocial stress findings

There is evidence that, like individuals with schizophrenia, those at elevated risk for the disorder due to clinical characteristics (i.e., UHR youth, individuals with SPD, and those reporting PLEs) are exposed to higher levels of psychosocial stress than healthy controls. However, it is not clear whether this is also true of individuals at elevated risk due to a family history of psychosis. However, both UHR youth and individuals with a family history of psychosis have been shown to exhibit greater emotional reactivity to stressful experiences than healthy controls. Across all samples, childhood trauma, distress relating to daily hassles, and perceived stress have been consistently shown to distinguish between high-risk individuals and healthy controls and are associated with the largest effect sizes. The evidence for major life events is less compelling, and the role of physical punishment requires further investigation. Whilst the temporal relationship between psychosocial stress exposure and psychosis vulnerability is unclear, prospective studies show that adolescents reporting PLEs are at greater risk of experiencing negative life events, physical assault, and bullying (concurrently and in the future). Exposure and reactivity to psychosocial stress has also been associated with psychotic symptoms among UHR youth, individuals with a family history of illness, and adolescents with SPD, and with depression and anxiety among UHR youth. Finally, longitudinal studies of high-risk individuals indicate that psychosocial stress is associated with an increase in psychotic symptoms over time and that sexual trauma specifically increases the risk for later transition to psychosis.

2.5 Cortisol

2.5.1 Cortisol in high-risk individuals compared to healthy controls

Studies comparing basal cortisol levels and cortisol responses elicited during psychosocial stressor tasks in individuals at elevated risk for schizophrenia and healthy controls are presented in Table 5 (ordered chronologically). Effect sizes comparing high-risk individuals and healthy controls on these measures are presented for comparison.

Individuals with a family history of illness

The systematic review identified six studies examining cortisol levels in relatives of patients with psychosis and healthy controls. All six studies examined adult first-degree relatives (age range: 18-55 years); two assessed cortisol responses to a metabolic stressor but were eligible for inclusion because they reported cortisol levels at baseline (Brunelin et al., 2008) or during the placebo condition (Marcelis et al., 2004); effect sizes were therefore computed from these data. Taken collectively, studies of individuals with a family history of illness have yielded inconsistent findings, which may be due to the different methodologies employed. Marcelis and colleagues measured fasting plasma cortisol levels at multiple time-points throughout the morning and observed higher cortisol levels in relatives compared to healthy controls; however, differences were small in magnitude and not statistically significant at any time-point (Marcelis et al., 2004). Three subsequent studies examining fasting plasma levels of cortisol at a single time-point in the morning (08:00 to 09:00 am) reported lower cortisol levels in relatives compared to healthy controls (Spelman et al., 2007; Brunelin et al., 2008; Yang et al., 2012); however, only one of these studies observed differences that approached statistical significance (Yang et al., 2012). In contrast, a study measuring serum cortisol levels in a non-fasting blood sample also obtained in the morning (08:00 am) reported significantly

higher cortisol levels among individuals with a family history of schizophrenia compared to controls (Yıldırım et al., 2011), although effect sizes were small. Similarly, a study using the ESM technique reported that relatives were characterised by moderately elevated salivary cortisol over multiple sampling events throughout the day compared to healthy controls (Collip et al., 2011).

Youth at ultra high-risk for psychosis

Two studies were identified that examined basal salivary cortisol levels in UHR youth (age range: 12-35 years), both of which observed higher cortisol levels among at-risk youth relative to healthy controls (Sugranyes et al., 2012; Walker et al., 2013). Despite the large effect size, however, the smaller of these studies only observed significant differences when UHR youth who were not receiving antipsychotic medication were compared to healthy controls (Sugranyes et al., 2012). Studies examining cortisol responses during psychosocial stressor tasks (i.e., experimental procedures designed to elicit an increase in cortisol) have yielded inconsistent findings. A small study of UHR youth (age range: 18-35 years) observed differences in the pattern of salivary cortisol secretion exhibited by at-risk youth and healthy controls during the Montreal Imaging Stress Task, assessing mental arithmetic ability (Mizrahi et al., 2012). Specifically, whilst healthy controls showed a decrease in salivary cortisol during the task, UHR youth showed no change. However, it is not clear whether the change in cortisol secretion during the task was significantly different between UHR youth and healthy controls (the study compared individuals with established schizophrenia, UHR youth, healthy controls, and only a significant main effect of group was reported), and insufficient data was provided to allow computation of effect sizes. In contrast, Pruessner and colleagues reported that UHR youth (age range: 16-27 years) completing the Trier Social Stressor Test, comprising both a mental arithmetic test and a public speaking task, showed lower levels of

salivary cortisol in the period prior to task commencement and lower levels of cortisol during the task compared to healthy controls (Pruessner et al., 2013a). Moreover, compared to the healthy control group, UHR youth showed a blunted cortisol response (i.e., a smaller increase in cortisol) within the first 10 minutes of the task. The divergent pattern of findings observed in these studies may relate to differences in the experimental procedures employed. In the former study (Mizrahi et al., 2012), participants performed the psychosocial stressor task while completed a positron emission tomography (PET) scan (a procedure which may in itself induce stress), whilst participants in the latter study (Pruessner et al., 2013a) completed the task under normal conditions.

Individuals with schizotypal personality disorder

Studies conducted by Walker and colleagues have shown that individuals with SPD are characterised by elevated cortisol levels. Neumann and Walker examined salivary cortisol samples obtained at multiple time-points throughout the day among adults with SPD (age range not reported; mean age: 41.0 years) and reported higher cortisol levels among these individuals compared to healthy controls at two of the time-points (15:00 and 16:00 pm), equating to moderate effect sizes (Neumann & Walker, 1999). However, mean cortisol levels across the day (averaged over time-points) were not significantly elevated. Subsequent studies by this group have used a similar cortisol sampling protocol (i.e., cortisol values averaged over multiple time-points throughout the day) but have examined adolescents meeting SPD criteria (age range: 12-18 years). The first of these studies reported that adolescents with SPD were characterised by elevated salivary cortisol relative to those with no personality disorder, equating to a moderate effect size (Weinstein et al., 1999). A subsequent study reported that the pattern and magnitude of differences was maintained at a two-year follow-up (Walker et al., 2001). A further study examining a larger sample

also observed elevated salivary cortisol in adolescents with SPD compared to those with no personality disorder (Mittal et al., 2007), albeit with a far smaller magnitude of difference. Consistent with these findings, a study assessing fasting plasma cortisol levels at multiple time-points throughout the day reported significantly elevated cortisol levels among adult psychiatric patients with SPD (age range not provided; mean age: 36.0) compared to healthy controls (Mitropoulou et al., 2004). In contrast, a small study examining groups of university students with schizotypal traits who presented with perceptual aberrations or with anhedonia reported that neither group showed differences in the total amount of salivary cortisol secreted during the Montreal Imaging Stress Task when compared to healthy controls (Soliman et al., 2011). Furthermore, there were no group differences in the proportion of individuals who exhibited an increase in cortisol during the task. Thus, whilst there is consistent evidence that individuals meeting SPD criteria are characterised by elevated cortisol, it is unclear whether this is also true of individuals presenting schizotypal traits.

Youth reporting psychotic-like experiences

Little work has been done to determine whether individuals reporting PLEs are characterised by abnormal cortisol levels. However, one recent study examined university students (age range not reported; mean age: 18.8 years) who obtained high scores on a questionnaire assessing hallucinations (Mittal et al., 2013). Compared to young adults with low levels of PLEs (scores in the bottom 10th percentile), those with high levels of PLEs (scores in the top 10th percentile) were characterised by elevated salivary cortisol levels, which, despite the small effect size, was statistically significant after adjusting for sampling time of day.

Table 5. Studies examining cortisol in high-risk individuals relative to healthy controls

Study	Sample	Age (mean)	Measure	Findings	*d*
Individuals with a family history of psychosis (FHx)					
Marcelis et al. (2004)	FHx (*n*=51) HC (*n*=50)	FHx=37.2 HC=35.0	Plasma cortisol levels (fasting) Multiple time-points (*t*=6): 10:00 am to 12:30 pm	No sig differences between FHx and HC on plasma cortisol levels during placebo condition at any time-point	Basal cortisol (range): 0.22 – 0.36
Spelman et al. (2007)	FHx (*n*=44) HC (*n*=38)	FHx=33.7 HC=25.2	Plasma cortisol levels (fasting) Single time-point: 08:00 am	FHx lower cortisol levels than HC although not sig different	Basal cortisol: -0.32
Brunelin et al. (2008)	FHx (*n*=15) HC (*n*=14)	FHx=28.5 HC=29.1	Plasma cortisol levels (fasting) Single time-point: 09:00 am	FHx lower cortisol levels than HC although not sig different	Basal cortisol: -0.58
Collip et al. (2011)	FHx (*n*=60) HC (*n*=63)	FHx=28.8 HC=33.3	Salivary cortisol levels Multiple time-points: ESM technique	FHx sig higher cortisol over sampling events (averaged) than HC	Mean cortisol: 0.45
Yıldırım et al. (2011)	FHx (*n*=70) HC (*n*=60)	FHx=39.3 HC=37.3	Serum cortisol levels Single time-point: 08:00 am	FHx sig higher cortisol levels than HC	Basal cortisol: 0.29
Yang et al. (2012)	FHx (*n*=32) HC (*n*=37)	FHx=27.6 HC=26.6	Plasma cortisol levels (fasting) Single time-point: 08:00 am	Trend for lower cortisol levels in FHx relative to HC	Basal cortisol: -0.45
Youth at ultra high-risk (UHR)					
Mizrahi et al. (2012)	UHR (*n*=12) HC (*n*=12)	UHR=23.0 HC=26.1	Salivary cortisol levels Multiple time-points: MIST	UHR no change in cortisol levels during MIST; cortisol decreased in HC	Insufficient data to compute

Note. HC: Healthy controls; sig: significant; ESM: Experience Sampling Method; MIST: Montreal Imaging Stress Task (Dedovic et al., 2005); *d*: standardised effect size derived from means; *t*: time-points.

Table 5. (continued)

Study	Sample	Age (mean)	Measure	Findings	*d*
Sugranyes et al. (2012)	UHR (*n*=33) [a] HC (*n*=13)	UHR=18.6 HC=20.3	Salivary cortisol levels Single time-point: 11:30 am	Trend for higher cortisol in total UHR compared to HC, sig for med-free UHR	Basal cortisol all UHR: 0.70 Medication-free UHR: 0.98
Pruessner et al. (2013a)	UHR (*n*=21) HC (*n*=21)	UHR=20.8 HC=20.8	Salivary cortisol levels Multiple time-points: TSST	UHR sig lower cortisol during TSST and blunted response relative to HC	Total cortisol secreted during TSST: -0.66
Walker et al. (2013)	UHR (*n*=256) HC (*n*=141)	UHR=19.0 HC=19.1	Salivary cortisol levels Multiple time-points (*t*=3): 10:00 am to 12:00 pm (values averaged)	UHR sig higher mean cortisol (averaged) than HC after adjusting for age, sampling time, and tobacco use	Mean cortisol: 0.24 *
Individuals with schizotypal personality disorder/schizotypal traits (SPD/ST)					
Neumann & Walker (1999)	SPD (*n*=17) HC (*n*=29)	SPD=41.0 HC=35.0	Salivary cortisol levels Multiple time-points (*t*=4): 13:00 pm to 16:00 pm	SPD sig higher cortisol than HC at 15.00 and 16.00 only, but not when values averaged over time-points	Cortisol at 15:00 pm: 0.60 Cortisol at 16:00 pm: 0.71 Mean cortisol: 0.26
Weinstein et al. (1999)	SPD (*n*=20) HC (*n*=26)	SPD=14.2 HC=13.9	Salivary cortisol levels Multiple time-points (*t*=4): 12:30 pm to 15:30 pm (values averaged)	SPD sig higher mean cortisol (averaged) than HC after adjusting for age	Mean cortisol: 0.59 **
Walker et al. (2001) [b]	SPD (*n*=12) HC (*n*=21)	Not provided	Salivary cortisol levels Multiple time-points (*t*=4): times not provided (values averaged)	SPD sig higher mean cortisol (averaged) than HC	Mean cortisol: 0.60

Note. HC: Healthy controls; sig: significant; TSST: Trier Social Stressor Test (Kirschbaum et al., 1993); *d*: standardised effect size derived from means; *t*: time-points. [a] 21/33 UHR medication-free, [b] study is a longitudinal follow-up (1.5–2.0 yrs) of the Weinstein et al. (1999) sample, age at follow-up not provided. * Computed from one-way ANCOVA statistics adjusted for age, sampling time, and tobacco use; ** computed from one-way ANCOVA statistics adjusted for age.

Table 5. (continued)

Study	Sample	Age (mean)	Measure	Findings	*d*
Mitropoulou et al. (2004)	SPD (*n*=15) HC (*n*=13)	SPD=36.0 HC=32.5	Plasma cortisol levels (fasting) Multiple time-points (*t*=7): 10:00 am to 12:00 pm (AUC computed)	SPD sig higher cortisol AUC values than HC during placebo (non-drug) condition	AUC cortisol: 0.92
Mittal et al. (2007)	SPD (*n*=39) HC (*n*=47)	SPD=14.2 HC=14.1	Salivary cortisol levels Multiple time-points (*t*=4/5): 09:00 am to 13:00 pm (values averaged)	SPD sig higher mean cortisol (averaged) than HC after adjusting for medication and age	Mean cortisol: 0.23
Soliman et al. (2011)	ST-p (*n*=12) ST-a (*n*=13) HC (*n*=15)	ST-p=19.5 ST-a=20.5 HC=19.6	Salivary cortisol levels Multiple time-points: MIST (AUC computed)	No sig differences between ST groups and HC on cortisol AUC values (total or increase)	Insufficient data to compute
Youth reporting psychotic-like experiences					
Mittal et al. (2013) [a]	High (*n*=34) Low (*n*=29)	High=18.8 Low=19.2	Salivary cortisol levels Multiple time-points (*t*=3): 09:00 am to 17:00 pm	High PLE group sig higher mean cortisol (averaged) than low PLE group (adjusting for sampling time)	Mean cortisol: 0.17 Cortisol AUC: 0.14

Note. HC: healthy controls; sig: significant; AUC: area under the curve; ST-p: schizotypal traits with perceptual aberrations; ST-a: schizotypal traits with anhedonia; MIST: Montreal Imaging Stress Task (Dedovic et al., 2005); *d*: standardised effect size derived from means; *t*: time-points. [a] Participants identified using the Launay–Slade Hallucination Scale - Revised (Bentall & Slade, 1985) assessing psychotic-like experiences; those scoring in the top and bottom 10th percentiles were eligible for high and low scoring groups respectively.

2.5.2 Associations between cortisol levels and current psychopathology

Several studies have investigated the extent to which cortisol levels are associated with psychopathology among UHR youth (age range: 12-35 years). One study of UHR youth reported that plasma cortisol levels were significantly correlated with anxiety and depression symptoms but were unrelated to positive and negative psychotic symptoms (Thompson et al., 2007). Consistent with these findings, two recent studies of UHR youth, found that salivary cortisol levels were not associated with prodromal symptoms (Corcoran et al., 2012; Sugranyes et al., 2012), with one study observing that cortisol was positively correlated with anxiety symptoms (Corcoran et al., 2012). However, data from the largest study of UHR youth indicated that salivary cortisol was significantly correlated with positive and negative psychotic symptoms (Walker et al., 2013), although correlation coefficients were small (0.13 and 0.09, respectively). Examining data over the total sample of adolescents with SPD, other personality disorders, and no personality disorders (age range: 12-18 years), salivary cortisol levels were significantly associated with negative but not positive psychotic symptoms in the initial study of SPD youth (Weinstein et al., 1999), and in the follow-up study, cortisol levels were associated with severity of SPD symptoms (Walker et al., 2001). Finally, using the ESM technique, a recent study reported that momentary increases in psychotic experiences were associated with increases in salivary cortisol among adult siblings of patients with psychosis (age range: 16-55 years) but not among controls (Collip et al., 2011).

2.5.3 Longitudinal studies relating cortisol levels to outcome

The few studies that have examined the extent to which cortisol is associated with symptom progression and diagnostic outcome have yielded inconsistent findings. A study of 29 UHR youth (age range: 14-30 years) reported significantly lower plasma cortisol levels among the five individuals who transitioned to psychosis within the

two-year follow-up period relative to youth who did not (Thompson et al., 2007). Similar findings were obtained in further study of UHR youth (age range: 14-30 years), although the lower salivary cortisol levels among those who developed psychosis were not significantly different from UHR youth who did not (Sugranyes et al., 2012). In contrast, a large study of UHR youth (age range: 12-35 years) reported that those who transitioned to psychosis had higher levels of salivary cortisol relative to those whose symptoms remitted, but not when compared to UHR youth who remained symptomatic or whose symptoms progressed (Walker et al., 2013). Salivary cortisol was also examined in a longitudinal study of high-risk adolescents with SPD and/or prodromal symptoms (age range: 12-18 years); those who developed psychosis during the one-year follow-up showed an increase in cortisol over time (Walker et al., 2010).

2.5.4 Summary of cortisol findings

Elevated basal salivary cortisol has been observed consistently among adolescents and young adults who are at elevated risk for psychosis on account of their clinical features. However, both lower and higher cortisol levels have been observed among individuals with a family history of psychosis, although this may be due to different methodologies employed across studies (i.e., fasting plasma samples vs. repeated saliva sampling). Furthermore, the extent to which individuals at elevated risk for schizophrenia show abnormal cortisol responses during psychosocial stressor tasks is unclear. There is limited evidence to suggest that cortisol levels are associated with psychotic symptoms among high-risk individuals. It is also currently unclear as to whether high-risk youth who transition to psychosis are characterised by elevated or decreased cortisol. One important limitation with the existing literature is that no study has as yet investigated whether high-risk individuals are characterised by a blunted cortisol awakening response relative to healthy controls.

2.6 Pituitary gland volume

2.6.1 Pituitary gland volume in high-risk individuals and healthy controls

Studies examining pituitary gland volume in individuals at elevated risk for schizophrenia and healthy controls are described in Table 6 (ordered chronologically) with effect sizes computed for comparison.

Individuals with a family history of illness

Only two studies have examined pituitary volume among individuals with a family history of psychosis. The first of these reported that pituitary volume was significantly larger among first-degree adult relatives of patients with psychosis in comparison to healthy controls (Mondelli et al., 2008), equating to a moderate-to-large effect size. Furthermore, the magnitude of difference between relatives and healthy controls was even larger among relatives of patients with familial schizophrenia (i.e., patients with first- or second-degree relatives with the disorder) compared to relatives of patients with non-familial schizophrenia. However, a subsequent study observed no differences in pituitary volume in adult siblings of individuals with psychosis and healthy controls (Habets et al., 2012). Whilst the studies did not differ in the ratio of males to females, relatives in the study by Mondelli and colleagues were often parents of patients and thus were typically older (mean age: 49.7 years) than those examined in the subsequent study (mean age: 28.3 years), which may account for the difference in findings.

Youth at ultra high-risk for psychosis

Neither of the studies examining pituitary volume in UHR youth reported data for the total UHR group (Garner et al., 2005; Büschlen et al., 2011), but instead examined pituitary volumes in relation to outcome status (i.e., psychosis onset) and compared healthy controls to UHR youth who had transitioned to psychosis and UHR youth

who had not. However, for both studies, data for the total UHR groups were provided in a recent meta-analysis (Nordholm et al., 2013). In the larger of these studies (Garner et al., 2005), pituitary volumes in the total UHR sample (age range: 14-30 years) were smaller relative to the healthy control group, but this difference was small in magnitude and not statistically significant. In contrast, Büschlen and colleagues observed larger pituitary volumes in the total UHR group compared to healthy controls (all participants aged 18 years or older), but while the effect was moderate in size, the difference did not reach statistical significance. Both study samples were similar in terms of sex, the proportion of medication-naive UHR youth within the sample, and subsequent transition rates; with the only difference being the inclusion of younger UHR youth (i.e., <18 years) in the study by Garner and colleagues, although mean ages were also similar across studies. Thus, the reason for the difference in findings is unclear.

Individuals with schizotypal personality disorder

Two studies were found to have examined pituitary volume in individuals with SPD. A study of young adults (age range not reported) observed that pituitary volumes were significantly larger among those with SPD relative to healthy controls (Takahashi et al., 2009), equating to a moderate effect size. In contrast, a recent study observed smaller pituitary volumes among individuals with SPD (age range: 18-55 years) compared to healthy controls, although the effect was only significant in males (Romo-Nava et al., 2013). Differences in findings may relate to illness severity and medication use; in the study by Takahashi and colleagues, all individuals with SPD were recruited from psychiatric services and the majority (85%) were receiving antipsychotic medication at the time of scanning. In contrast, individuals with SPD in the study by Romo-Nava and colleagues were recruited from the community and all were medication-naive.

Table 6. Studies examining pituitary gland volume in high-risk individuals relative to healthy controls

Study	Sample	Age (mean)	Measure	Findings	*d*
Individuals with a family history of psychosis (FHx)					
Mondelli et al. (2008)	FHx (*n*=44) HC (*n*=46)	FHx=49.7 HC=39.7	Manual tracing using ANALYZE software	FHx sig larger pituitary volume than HC (adjusted for age, WBV, and sex)	Pituitary volume: 0.70
Habets et al. (2012)	FHx (*n*=37) HC (*n*=32)	FHx=28.3 HC=32.9	Semi-automated (manual tracing and automatic tissue classification) using GIANT software	Pituitary gland volumes identical in FHx and HC (adjusted for ICV)	Pituitary volume: 0.00
Youth at ultra high-risk (UHR)					
Garner et al. (2005)	UHR (*n*=94) HC (*n*=49)	UHR=19.8 HC=20.2	Manual tracing using ANALYZE software	No sig difference in pituitary volume in UHR vs. HC (adjusted for WBV)	Pituitary volume: -0.11*
Büschlen et al. (2011)	UHR (*n*=36) HC (*n*=20)	UHR=24.9 HC=22.9	Manual tracing using AMIRA software	UHR larger pituitary volume than HC but not sig (adjusted for WBV)	Pituitary volume: 0.45*
Individuals with schizotypal personality disorder (SPD)					
Takahashi et al. (2009)	SPD (*n*=47) HC (*n*=81)	SPD=25.0 HC=24.5	Information not provided	SPD sig larger pituitary volume than HC (adjusted for age and ICV)	Pituitary volume: 0.47
Romo-Nava et al. (2013)	SPD (*n*=40) HC (*n*=67)	SPD=29.4 HC=30.2	Manual tracing using 3DSlicer software	SPD sig larger pituitary volume than HC in males only (adjusted for age, education, SES, and WBV)	Pituitary volume (males only): -0.77

Note. HC: Healthy controls; sig: significant; WBV: whole-brain volume; ICV: intracranial volume; SES: socioeconomic status; *d*: standardised effect size derived from means * Insufficient data provided, means and SD obtained from a meta-analysis (Nordholm et al., 2013) reporting raw data provided by the study authors.

2.6.2 Associations between pituitary volume and current psychopathology

Whilst only three studies of high-risk individuals have examined the relationship between pituitary volume and psychopathology, consistent results have emerged indicating a lack of association. Pituitary volume was not significantly correlated with positive or negative symptoms in the clinically-derived sample of patients with SPD (Takahashi et al., 2009), or in the largest study of UHR youth published to date (Garner et al., 2005), which also observed no relationship between pituitary volume and symptoms of anxiety or depression. Consistent with these findings, among adult relatives of patients with schizophrenia, schizotypal symptoms were not significantly correlated with pituitary volume (Mondelli et al., 2008).

2.6.3 Longitudinal studies relating pituitary volume to outcome

In contrast to the inconsistent pattern of findings observed when all UHR youth were compared with healthy controls (i.e., both smaller and larger volumes reported among UHR youth relative to controls), both studies examining the relationship between pituitary volume and transition to psychosis among UHR youth have yielded similar results. Specifically, both studies observed that UHR youth who later developed psychosis (participants followed for a minimum of one year and up to 3.7 years) had larger pituitary volumes at baseline than both UHR youth who did not develop psychosis and healthy controls (Garner et al., 2005; Büschlen et al., 2011). Furthermore, Garner and colleagues observed that larger pituitary volume was highly predictive of illness onset. The only study of individuals at elevated risk for schizophrenia to have examined changes in pituitary volume over time observed a significant increase in pituitary volume among patients with SPD during a three-year follow-up that was not present in the healthy control group (Takahashi et al., 2011). However, changes in pituitary volume were not significantly associated with symptom progression among individuals with SPD.

2.6.4 Summary of pituitary volume findings

Studies comparing pituitary gland volume in individuals at elevated risk for schizophrenia with that of healthy controls have yielded conflicting findings. Whilst there is some evidence to suggest that individuals with SPD, and less consistently, relatives of individuals with schizophrenia, are characterised by abnormal pituitary volumes relative to healthy controls, both larger and smaller volumes have been reported. However, the only two studies of UHR youth published to date have reported similar findings. These studies indicate that whilst pituitary volumes across the total sample of UHR youth do not differ from healthy controls, those who later transition to psychosis show enlarged pituitary volumes compared to both UHR youth who do not transition and healthy controls. The few studies which have examined the relationship between pituitary volume and psychopathology have also yielded consistent negative findings, with no study of high-risk individuals having observed an association between pituitary volume and psychotic symptoms.

2.7 Summary of findings across domains

Table 7 presents a summary of the findings of the systematic review. As illustrated, there is evidence that youth at UHR and individuals with SPD are characterised by increased susceptibility to psychosocial stress (higher levels of exposure and greater reactivity to these exposures) and elevated cortisol levels. Whilst there have been fewer studies of individuals reporting PLEs, similar findings have been observed in this group. In contrast, there is no evidence that individuals with a family history of schizophrenia are more likely to experience psychosocial stress, although increased stress reactivity has been reported, and both higher and lower cortisol levels have been observed in this population. The extent to which high-risk individuals are characterised by abnormal pituitary volume is unclear, with enlarged volumes, volume reductions, and no volume differences all reported relative to controls.

Table 7. Summary of psychosocial stress, cortisol, and pituitary volume abnormalities across high-risk groups

Domain	Individuals with a family history of schizophrenia	Youth at ultra high-risk for psychosis	Individuals with schizotypal personality disorder	Individuals reporting psychotic-like experiences
Psychosocial stressor exposure	–	↑	↑	↑
Psychosocial stress reactivity	↑	↑	↑	Not examined
Cortisol levels	↓ ↑	↑	↑	↑
Pituitary volume	↑	–	↓ ↑	Not examined

Note. ↑ Increased in high-risk group relative to healthy controls; ↓ decreased in high-risk group relative to healthy controls; – no difference between high-risk group and healthy controls. Psychosocial stress reactivity: emotional reactivity to stressful events as assessed using the Experience Sampling Method or distress ratings relating to other psychosocial stress exposures (e.g., daily hassles and negative life events); cortisol levels: basal cortisol levels (as measured in plasma, serum, or saliva) or cortisol responses during psychosocial stressor tasks.

2.8 Methodological considerations

2.8.1 Limitations associated with previous studies of high-risk individuals

Influence of different vulnerability profiles for schizophrenia

The neural diathesis-stress model proposes that underlying biological vulnerability to psychosis influences the extent to which psychosocial stress increases the risk for subsequent illness (Walker & Diforio, 1997; Walker et al., 2008). However, it is not currently clear whether individuals with a putative genetic predisposition to develop schizophrenia (as conferred by a family history of illness) and those with clinical vulnerability factors are similarly affected by psychosocial stressors or whether they show the same HPA axis abnormalities. Whilst studies of UHR youth, adolescents with SPD, and youth presenting PLEs demonstrate that these individuals experience higher levels of exposure and reactivity to psychosocial stressors and show elevated cortisol levels relative to healthy controls, studies investigating these characteristics in individuals with a family history of psychosis have yielded inconsistent findings. Furthermore, the extent to which relatives of psychosis patients in some of these studies remain at elevated risk for schizophrenia is unclear, as many have passed through the typical age period of peak illness onset (~30 years). No study has yet compared experiences of psychosocial stress or HPA axis function in different groups of high-risk individuals; thus, further studies examining youth with different vulnerability profiles are needed. The current study will address these limitations by examining children at putatively elevated symptomatic-risk for schizophrenia who present multiple antecedents of the disorder, and those at genetic-risk due to a family history of illness. However, as noted previously, the distinction may be somewhat artificial (i.e., children at symptomatic-risk may also possess schizophrenia susceptibility genes, and any psychosocial stress or HPA axis abnormalities observed among children at elevated genetic-risk may be due to environmental factors).

Concurrent measures of stress and HPA axis function

The HPA axis provides a potential mechanism by which psychosocial stress might influence the structure and function of the brain and thus lead to psychosis onset (Walker & Diforio, 1997; Walker et al., 2008). However, few studies have examined the association between experiences of psychosocial stress and HPA axis function among individuals at elevated risk for schizophrenia. Higher plasma cortisol levels were correlated with the number of daily hassles (but not with the number of recent life events) in a study of UHR youth (Thompson et al., 2007). Furthermore, a study employing the ESM technique demonstrated that stressful events and emotional reactivity to these events were associated with increases in salivary cortisol in relatives of psychosis patients but not healthy controls (Collip et al., 2011). Finally, three studies of UHR youth reported that salivary cortisol levels were positively associated with clinician-rated impaired stress tolerance (Corcoran et al., 2012; Sugranyes et al., 2012; Walker et al., 2013). Thus, whilst there is some evidence to suggest that the cortisol abnormalities observed among high-risk individuals are due to increased psychosocial stress exposure and/or increased reactivity to these stressors, further studies are needed to confirm this finding.

In contrast, there is limited evidence to suggest that the pituitary volume abnormalities which have been observed among individuals at elevated risk for schizophrenia reflect stress-induced HPA axis hyperactivity. A study of UHR youth reported that plasma cortisol levels were not significantly correlated with pituitary volume (Thompson et al., 2007). Consistent with this finding, the only other study to have measured cortisol levels and pituitary volume concurrently observed no association between these measures among young adults with a family history of psychosis (Habets et al., 2012). Whilst no study of high-risk individuals has yet examined the relationship between pituitary volume and stress exposure, Habets and

colleagues also reported that pituitary volumes among patients with psychosis, and to a lesser extent, their adult siblings, were positively associated with emotional stress reactivity (Habets et al., 2012). Even within healthy populations, few studies have examined the relationship between pituitary volume and psychosocial stress exposure. However, studies of children with PTSD (Thomas & De Bellis, 2004) and adolescents with borderline personality disorder (Garner et al., 2007) have observed pituitary volume abnormalities among those exposed to childhood maltreatment. Thus, further studies employing concurrent measures of psychosocial stress and HPA axis function are needed. This thesis, which examines children at elevated risk for schizophrenia, will use measures of psychosocial stress, cortisol levels, and pituitary volume collected at the same assessment phase in order to determine the relationship these factors.

Relationship to neurocognitive function

Neurocognitive deficits are a core feature of schizophrenia (Kahn & Keefe, 2013) and have been consistently observed among individuals at elevated risk for the disorder (Siever & Davis, 2004; Fusar-Poli et al., 2012b; Agnew-Blais & Seidman, 2013). Impairments in memory and executive function are particularly pronounced among individuals with established illness (Reichenberg & Harvey, 2007), and several lines of evidence suggest that stress-induced HPA axis dysfunction may contribute to these deficits. Firstly, animal studies indicate that persistently-elevated glucocorticoid levels and behavioural stressors can cause damage to hippocampal cells and structural changes in the medial prefrontal cortex (Sapolsky, 2000; Corcoran et al., 2003; Cerqueira et al., 2008). Secondly, among individuals with psychosis, elevated cortisol has been associated with memory and executive function deficits (Walder et al., 2000), and a greater blunting of the CAR has been found to correlate with poorer verbal memory (Aas et al., 2011b). Finally, studies of individuals with schizophrenia

have reported that childhood maltreatment is associated with greater impairments in memory and executive function (Lysaker et al., 2001; Shannon et al., 2011); although similar results have not been obtained other studies (Schenkel et al., 2005; Aas et al., 2011b; Sideli et al., in press). To date, no study of individuals at elevated risk for schizophrenia has yet investigated whether the neurocognitive deficits that characterise these individuals are associated with experiences of psychosocial stress and HPA axis dysfunction. The current study will be the first to investigate the relationship between neurocognitive function and experiences of psychosocial stress and cortisol levels among children at elevated risk for schizophrenia. This study will also examine whether the same relationship exists among healthy children.

2.8.2 Additional factors of relevance to the current study

Several potentially confounding factors (discussed below) may obscure group differences in experiences of psychosocial stress and HPA axis function.

Adolescence, puberty, and sex

Adolescence is typically a time of marked developmental change characterised by increases in sex hormones and changes in affect and behaviour (Buchanan et al., 1992). Evidence from cross-sectional and longitudinal studies indicates that there are both age- and puberty-related effects on cortisol levels which increase between childhood and adulthood (Gunnar & Vazquez, 2006). Furthermore, among adolescents, more advanced pubertal stages have been associated with a decreased CAR (Adam, 2006), and increasing age associated with a more blunted cortisol response to stress (Gunnar et al., 2009). Consistent with these findings, pituitary volume also increases during adolescence and is correlated with pubertal stage (Elster et al., 1990; Zipursky et al., 2011). In adolescent populations there also appears to be a sex difference in salivary cortisol levels during the morning (Netherton et al., 2004; Rosmalen et al., 2005), with girls showing higher levels than

boys. Additionally, female adolescents have larger pituitary volumes than males (Elster et al., 1990; MacMaster et al., 2007b), a pattern that has also been observed consistently in studies of adults with psychosis (Nordholm et al., 2013).

Ethnicity and socioeconomic status

There is also evidence to suggest that ethnicity and socioeconomic status may influence HPA axis function. A large study of adults reported that those of low socioeconomic status and black ethnicity had higher cortisol during the evening relative to individuals of high socioeconomic status and white ethnicity, respectively (Cohen et al., 2006). Similarly, young children from low socioeconomic backgrounds have been found to show elevated daytime cortisol compared to those of high socioeconomic status (Lupien et al., 2001). Additionally, a large study of adolescents reported a U-shaped association between socioeconomic status and morning cortisol levels, with those from high and low socioeconomic families showing a blunted CAR relative to adolescents from intermediate families (Marsman et al., 2012). Ethnic differences have also been observed in cortisol responses to psychosocial stress, specifically, individuals of black ethnicity have been reported to show a blunted cortisol response relative to white participants (Chong et al., 2008). It is possible that ethnic differences in basal cortisol levels and cortisol reactivity may reflect differences in psychosocial stress exposure. For example, a recent North American study reported that among UHR youth, those from ethnic minority groups (African-American, Hispanic, and Asian) were more likely to have experienced trauma (particularly physical abuse) than white youth (Thompson et al., 2009). Similarly, a study of first-episode psychosis patients and healthy controls in the UK observed that individuals of black Caribbean ethnicity were more likely to have experienced long-term parental separation than white British participants (Morgan et al., 2007).

Tobacco and substance use

Long-term tobacco use may also affect HPA axis function. Smokers have been shown to exhibit a blunted cortisol response to psychosocial stress (Rohleder & Kirschbaum, 2006) and an altered CAR relative to non-smokers (Clow et al., 2004; Fries et al., 2009). Whilst acute cannabis administration has been associated with elevated cortisol (D'Souza et al., 2004), little work has been conducted to determine the effect of long-term cannabis use on the HPA axis. However, a large study of adolescents recently reported that cannabis users exhibited a blunted cortisol response to stress relative to non-users (van Leeuwen et al., 2011).

2.9 Conclusions

Consistent with a previous systematic review examining psychosocial stress and HPA axis function among high-risk individuals (Aiello et al., 2012), the current review indicates that both individuals with a family history of illness and UHR youth show increased reactivity to psychosocial stressors, and additionally demonstrates that this feature also characterises individuals with SPD. The current review also concludes that individuals at elevated risk for schizophrenia on account of their clinical features (i.e., UHR youth, individuals with SPD, and those reporting PLEs) are exposed to higher levels of psychosocial stressors (e.g., major life events, daily hassles, and trauma) relative to healthy controls. Further work is merited to establish whether exposures such as physical punishment are also more prevalent among high-risk individuals. Aiello and colleagues reported that UHR youth, but not individuals with a family history of illness, exhibit elevated cortisol levels during the day. The current review concurs with this conclusion, and also demonstrates that individuals with SPD and those reporting PLEs are similarly characterised by elevated cortisol relative to healthy controls. However, the extent to which high-risk individuals also exhibit a blunted CAR is not yet known. In contrast to previous

reviews (Aiello et al., 2012; Nordholm et al., 2013), the current review found no consistent evidence to indicate that that high-risk individuals are characterised by pituitary volume abnormalities. Whilst abnormal pituitary volumes have been observed in some studies of adult relatives of individuals with psychosis and individuals with SPD, other studies have reported no differences between high-risk individuals and controls. However, there is evidence that UHR youth who later transition to psychosis show increased pituitary volume relative to UHR youth who do not transition and healthy controls. The current review included several studies examining pituitary volume that were not included in previous reviews, which is likely to have contributed to the difference in findings.

The studies examined in this review suggest that HPA axis abnormalities, potentially caused by exposure to psychosocial stress, are present prior to the onset of psychosis and may increase the risk of subsequent illness. Unfortunately, several characteristics of the high-risk samples examined to date limit the ability to draw this conclusion. Firstly, many of the studies of individuals with a family history of schizophrenia have included relatives who have passed through the typical age period of peak illness onset (~30 years); thus, the extent to which these individuals remain at elevated risk for the disorder is unclear. Secondly, UHR youth are by definition already sufficiently distressed as to seek treatment for their symptoms, and therefore, elevations in cortisol may be due to emerging psychosis rather than psychosocial stressor exposure. Finally, studies of youth with SPD, although not typically confounded by help-seeking behaviour, are limited by the fact that these individuals already present with symptoms of sufficient severity as to meet diagnostic criteria for a schizophrenia spectrum disorder. In addition to these limitations, no study has directly compared individuals with a family history of psychosis (with putatively genetically-mediated risk) those at symptomatic risk for

psychosis, and few have assessed psychosocial stress and HPA axis function concurrently. Furthermore, the extent to which neurocognitive function among high-risk individuals is associated with experiences of psychosocial stress and HPA axis function has yet to be investigated. The work presented in Chapters 4, 5, 6, and 7 aims to address some of these methodological limitations by:

- Examining exposure and reactivity to psychosocial stressors, and biological markers of HPA axis function in children at elevated risk for schizophrenia, none of whom are yet seeking treatment for their symptoms.
- Comparing children with different vulnerability profiles for schizophrenia, that is, children at symptomatic-risk who present multiple antecedents of the disorder, and those at genetic-risk due to a family history of illness.
- Assessing both exposure to psychosocial stress and distress relating to these exposures in children at elevated risk for schizophrenia, and additionally exploring the association between risk status and physical punishment.
- Examining the CAR and diurnal cortisol in high-risk children and the extent to which these measures relate to experiences of psychosocial stress.
- Measuring pituitary volume in medication-naive high-risk children and examining the relationship with cortisol levels and psychosocial stress.
- Exploring the extent to which neurocognitive function among children at elevated risk for schizophrenia is associated with experiences of psychosocial stress and cortisol levels.
- Examining the role of additional factors (e.g., age, puberty, sex, socioeconomic status, ethnicity, and substance use) that may influence the relationship between risk status and psychosocial stress/HPA axis function.

CHAPTER 3 Study methodology

3.1 Introduction

This thesis examines experiences of psychosocial stress and HPA axis function among children recruited to the London Child Health and Development Study (CHADS; http://www.chads-project.org.uk), a longitudinal investigation of children at putatively elevated risk for psychosis because they present multiple antecedents of schizophrenia (ASz) or a family history of illness (FHx). The study also follows the development of a group of low-risk typically-developing (TD) children. The study was established in 2005 by researchers at the Institute of Psychiatry, including Dr Kristin Laurens (Principal Investigator) and Professors Sheilagh Hodgins, Barbara Maughan, Robin Murray, and Eric Taylor. The overall aim of the study was to develop a novel method of identifying children within the general population who are at elevated risk for developing schizophrenia. The work was primarily supported by funding received by Dr Kristin Laurens from the National Institute for Health Research (NIHR).

Chapter aims

This chapter describes the methodology of the CHADS project; the specific aims of this chapter are as follows:

1. Provide a detailed description of the procedures used to recruit ASz, FHx, and TD children to the CHADS project.

2. Delineate the assessment phases of the study and compare the three groups on the extent to which they completed the measures examined in this thesis.

3. Describe the measures used to assess sociodemographic characteristics and current psychopathology.

4. Outline the analytic strategy employed in subsequent chapters.

3.2 Participant recruitment

3.2.1 Recruitment of ASz and TD children

ASz and TD children aged 9-12 years were recruited using a novel, cost-effective, community-screening method (Laurens et al., 2007; Laurens et al., 2011). The screening procedure was conducted in 73 primary schools in the Greater London area (predominately located in inner-city boroughs) using an 'opt-out' procedure. Specifically, after obtaining approval from the head of the school, children were provided with a letter for their caregiver that described the content of the screening questionnaire and the scheduled date of screening. Caregivers who did not wish their child to participate in the screening procedure (4%) were asked to indicate their refusal on a form to be returned to the school. Collaborating schools were sampled to reflect the range of socioeconomic disadvantage represented in London schools (as indexed by the percentage of children eligible to receive free school meals) and included both state and religious schools. Children completed screening questionnaires in class in the presence of a researcher who read the questions aloud and ensured that children responded independently. Each child was subsequently issued with a corresponding questionnaire for their primary caregiver who was asked to return the completed form via reply-paid mail. Screening questionnaires included items assessing the antecedent triad (see Appendices), defined as (i) a delay or abnormality in speech and/or motor function, (ii) a social, emotional, and/or behavioural problem, and (iii) a psychotic-like experience. The measures used to assess the antecedent triad are described in detail below. ASz children were those who presented with abnormalities in each of the three antecedent domains. The TD group comprised children who presented none of antecedents and who had no first-, second-, or third-degree relatives with a schizophrenia-spectrum disorder as confirmed using the Family Interview for Genetic Studies [FIGS (Maxwell, 1992)].

Delays and abnormalities in speech and/or motor function

The caregiver questionnaire included nine items assessing delays or abnormalities in speech or motor development (Laurens et al., 2007). Three quantitative questions were used to ascertain the age at which speech and motor developmental milestones were attained (using a selection of age range options); delayed milestone attainment was defined according to the World Health Organisation population norms (age > 95th percentile reported for milestone attainment). Five qualitative (yes/no) questions enquired as to whether there were any parental or professional concerns regarding speech or motor development, and one further qualitative question assessed whether the child had experienced any difficulties with coordination or unsteadiness. The presence of a speech and/or motor delay or abnormality was defined as at least one caregiver-reported problem among these nine items.

Social, emotional, and behavioural problems

The child and caregiver questionnaires included items from the Strengths and Difficulties Questionnaire [SDQ (Goodman, 2001)], a well-established measure of child psychopathology with demonstrated validity for identifying children with mental health problems. The measure shows moderate-to-high internal and test-retest reliabilities for the parent- and self-report versions (Goodman, 2001; Goodman et al., 2003) and can be reliably completed by children as young as eight years (Muris et al., 2004). The SDQ includes four psychopathology scales: emotional problems, conduct problems, hyperactivity-inattention, and peer relationship problems. Each scale includes five items rated 0 'not true', 1 'somewhat true', or 2 'certainly true', yielding a total score of 10 for each scale. The SDQ has been evaluated extensively in UK population samples and three normative bandings are provided: 'normal' (scores below the 80th percentile on UK population norms), 'borderline' (scores within the 80th to 90th percentiles), and 'abnormal' (scores above the 90th percentile). A social,

emotional, and/or behavioural problem was defined as a score in the 'abnormal' range on at least one of the four psychopathology scales. Caregiver-report was used for all scales with the exception of the emotional problems scale based on evidence that child reports of internalising symptoms may be more accurate than parent reports (Edelbrock et al., 1986; Angold et al., 1987).

Psychotic-like experiences (PLEs)

The child questionnaire included nine PLE items (Table 8) assessing a range of hallucination- and delusion-like experiences (Laurens et al., 2007; Laurens et al., 2012). Five items were adapted from the Diagnostic Interview Schedule for Children [DISC (Costello et al., 1982)], including, auditory hallucinations, thoughts read, ideas of reference, paranoid ideas, and ideas of somatic changes. These five items from the DISC were used in the Dunedin study and have previously been shown to distinguish between children who develop schizophreniform disorder by age 26 years and those who do not (Poulton et al., 2000). Four additional items were included to capture a broader range of experiences, including, visual hallucinations, passivity phenomena, telepathic experiences, and grandiosity. Each item was rated on a three-point scale: 0 'not true', 1 'somewhat true', or 2 'certainly true'. For the purposes of identifying children who present the triad of antecedents, the presence of a PLE was defined as at least one child-reported 'certainly true' experience. A recent study examining the psychometric properties of this measure in a general population sample of nearly 8,000 children aged 9-12 years reported that the nine PLE items load onto a single latent psychotic-like construct that is distinct from, although correlated with, internalising and externalising symptom dimensions (Laurens et al., 2012). A similar seven-item screening questionnaire has been shown to exhibit adequate criterion validity for clinician-rated subclinical psychotic symptoms reported at interview in a sample of children aged 11-13 years (Kelleher et al., 2011).

Table 8. Psychotic-like experiences assessed in the screening questionnaire

Symptom domain	Item
Thoughts read [a]	"Some people believe that their thoughts can be read. Have other people ever read your thoughts?"
Ideas of reference [a]	"Have you ever believed that you were being sent special messages through the television?"
Paranoid ideas [a]	"Have you ever thought that you were being followed or spied upon?"
Auditory hallucinations [a]	"Have you ever heard voices that other people could not hear?"
Somatic changes [a]	"Have you ever felt as though your body had been changed in some way that you could not understand?"
Passivity phenomenon	"Have you ever felt that you were under the control of some special power?"
Telepathic experiences	"Have you ever known what another person was thinking even though that person wasn't speaking?"
Grandiosity	"Do you have any special powers that other people don't have?"
Visual hallucinations	"Have you ever seen something or someone that other people could not see?"

Note. [a] Items adapted from the Diagnostic Interview Schedule for Children (Costello et al., 1982). Each item was scored as 0 'not true', 1 'somewhat true', or 2 'certainly true'.

3.2.2 Recruitment of FHx children

Two methods were used to identify children with a family history of schizophrenia. Firstly, the caregiver screening questionnaire included items to assess family mental health difficulties (i.e., caregivers were asked whether any family members had experienced mental health problems and to indicate the nature of these problems). Secondly, the medical records of mental health service users within the South London and Maudsley (SLaM) National Health Service (NHS) Foundation Trust were reviewed to identify patients with a diagnosis of schizophrenia or schizoaffective disorder who had a child relative aged between 9 and 12 years. Identified families were approached following liaison with the patient's care worker. The presence of a family history of schizophrenia was subsequently confirmed using the FIGS (Maxwell, 1992), a semi-structured interview completed with the caregiver which assesses mental health problems among family members. FHx children (identified either via community screening or via medical records) had at least one first- or second-degree relative with schizophrenia or schizoaffective disorder.

3.2.3 Exclusion criteria

The following exclusion criteria were applied to all groups:

(i) Insufficient English language ability in the child or caregiver to complete assessments.

(ii) The presence of a neurological condition in the child that affected developmental milestone attainment or current functioning (e.g., epilepsy or cerebral palsy).

(iii) A diagnosis of autism or Asperger's disorder in the child or a diagnosed learning disability (defined as an IQ score < 70).

(iv) Child having previously experienced a psychotic episode or having ever received antipsychotic medication.

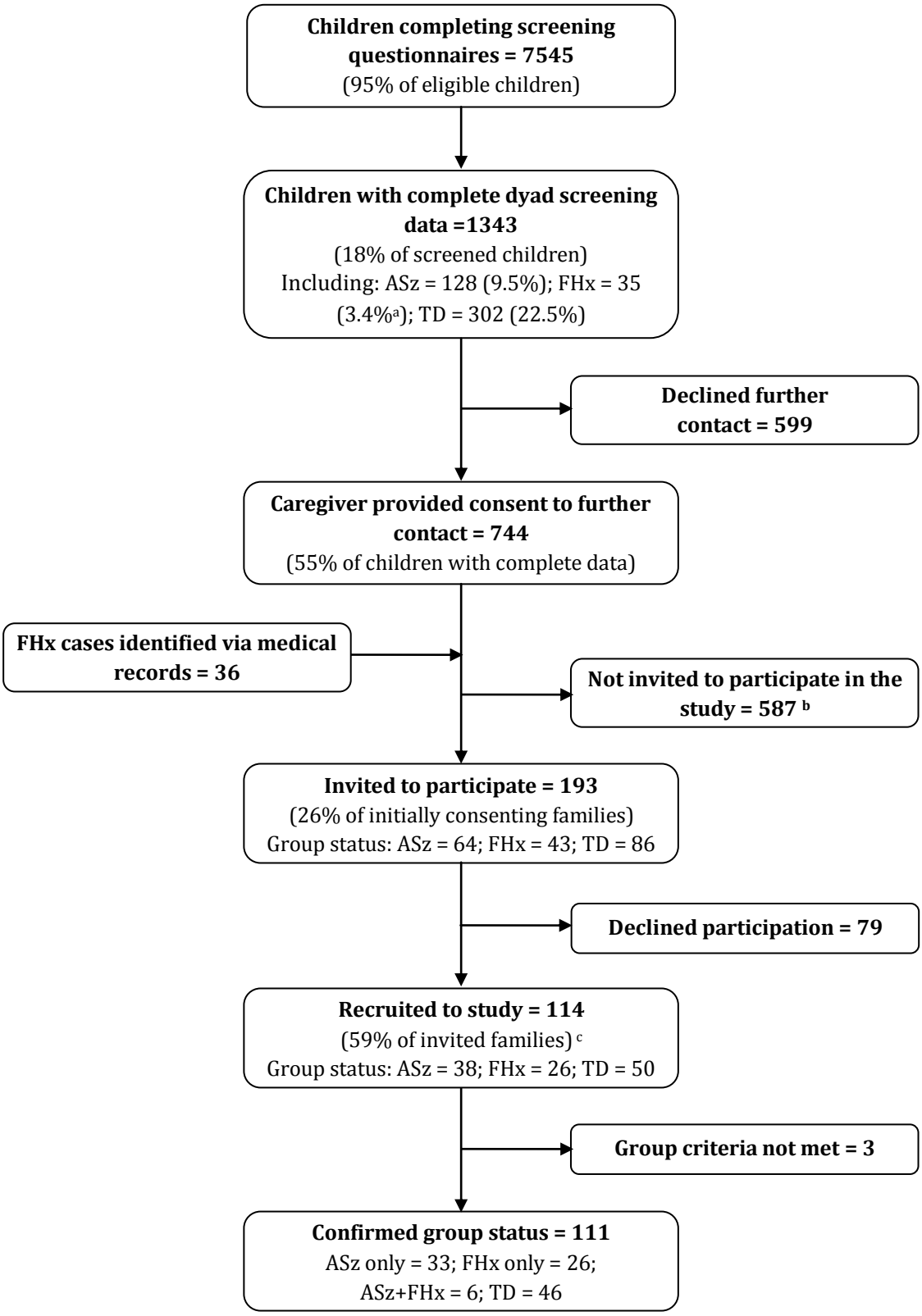

Figure 5. Recruitment pathway

Note. ASz: all three antecedents; FHx: reported family history of schizophrenia or schizoaffective disorder; TD: none of the antecedents and no family history of schizophrenia/schizoaffective disorder. [a] Denominator = 1020 (family history items not included in the first wave of screening). [b] Eligibility criteria not met or recruitment not logistically feasible (e.g., invalid contact details). [c] Recruitment rates among potential ASz, TD, and FHx: 59%, 60%, and 58% respectively.

3.2.4 Recruitment rates

Participant flow through the study is summarised in Figure 5. Complete screening data (i.e., child- and caregiver-report) were available for 1,343 children. Among these children, 9.5% presented all three antecedents, 3.4% were reported to have a family history of schizophrenia/schizoaffective disorder, and 22.5% presented none of the antecedents. These children were potentially eligible for the ASz, FHx, and TD groups, respectively. Half of the families supplying child- and caregiver-questionnaires (55%) agreed to be contacted regarding further research. Permission was obtained to contact a further 36 families who were identified via medical records (i.e., 36 SLaM service users with a diagnosis of schizophrenia agreed for their child relatives to be approached regarding the study). In total, 193 families (26% of those who indicated their willingness to receive information about the study) were subsequently invited to participate in a longitudinal study of child development; the remaining children either did not meet eligibility criteria or it was no longer logistically feasible to recruit these cases (e.g., contact details for the family had changed or the family had moved out of the area). Of those invited to participate, 114 (59%) were subsequently recruited to the study; participation rates for those who were potentially eligible for the ASz, FHx, and TD groups were 59%, 60%, and 58%, respectively. Following the initial assessment session to confirm group status (i.e., when all families completed the FIGS, and antecedent questionnaires were completed for potential FHx children identified via medical records), 33 children met ASz criteria only, 26 met FHx criteria only, 6 met both ASz and FHx criteria, and 46 met TD criteria.

Independent samples t-tests and chi-squared tests were used to compare the characteristics of ASz and TD children who participated in the study and children who were potentially eligible for these groups but did not participate for one of the following reasons: (i) their caregivers provided questionnaire data but did not supply

contact information to receive an invitation for further research, (ii) their caregivers supplied contact information but no further contact with the family was established, or (iii) caregivers/children declined their invitation to participate further. Compared to TD children who did not participate in the study, TD participants did not differ on age, sex, or ethnicity (p>0.05). Furthermore, there were no significant group differences between TD participants and TD non-participants in the proportion of children who presented with scores in the 'borderline' range on each of the four SDQ psychopathology scales or the proportion of children reporting at least one 'somewhat true' PLE. Among potential ASz cases, those who participated in the study did not differ to ASz non-participants on age, sex, ethnicity, or the prevalence of triad components (i.e., the proportion of children obtaining SDQ scores in the 'abnormal' range or the proportion of children reporting at least one 'certainly true' PLE), with the exception that ASz participants were less likely to score in the 'abnormal' range on the SDQ emotional symptoms scale than ASz non-participants (p=0.01).

3.2.5 Cases meeting ASz and FHx criteria

As described above, six children met both ASz and FHx criteria. Specifically, three children eligible for recruitment to the ASz group were subsequently confirmed to have a first- or second-degree relative with schizophrenia or schizoaffective disorder when the FIGS was completed with their caregiver. Similarly, three children who were recruited via medical records as relatives of individuals with schizophrenia were found to present the triad of antecedents when they completed the antecedent screening questionnaires. In order to most accurately reflect the ASz and FHx populations from which participants were sampled, these six ASz+FHx children were retained in both groups yielding total group sizes of 39 ASz children and 32 FHx children. Given the non-independent nature of the resultant ASz and FHx groups, all analyses reported in this thesis compare each at-risk group to the TD group only.

3.3 Procedure

Children meeting ASz, FHx, and TD criteria were invited to participate in a longitudinal study of child development conducted at the Institute of Psychiatry, King's College London. Ethical approval for each phase of the study was granted by the Joint SLaM and Institute of Psychiatry NHS Research Ethics Committee. Caregivers and children provided written informed consent and assent, respectively, for participation.

3.4 Follow-up assessment phases

Assessment phases were completed at approximately two-yearly intervals commencing at age 9-12 years (baseline) with follow-ups at 11-14 years (24-month) and 13-16 years (48-month). A range of biological and psychosocial assessments were completed by children and their caregivers at each assessment phase. This thesis primarily examines cross-sectional data on psychosocial stress, salivary cortisol, and pituitary volume collected at the 24-month follow-up assessment. Children who did not participate in the 24-month assessment phase, or who were unable to complete the measures examined in this thesis, were re-approached at the 48-month follow-up phase. For example, some children were unable to complete an MRI scan at the 24-month assessment due to the presence of contraindicators (e.g., metal dental braces) but completed a scan two years later. Data obtained at the 48-month assessment phase were included in this thesis for children who were still within the 24-month age range (i.e., 11-14 years) at the time of their 48-month assessment, and are herein referred to as 24-month assessment data.

Of the 111 ASz, FHx, and TD children recruited to the CHADS project, 99 (89%) were followed-up approximately 24-months later and completed at least one of the three measures examined in this thesis. Specifically, 95 children (86%) completed psychosocial stress measures, 91 (82%) provided salivary cortisol data, and 79

(71%) completed a structural MRI scan which was used to examine pituitary volume. Table 9 indicates the follow-up rates by group; among ASz, FHx, and TD children, 90%, 78%, and 96%, respectively, participated in the 24-month assessments. Statistical analyses confirmed that ASz and TD groups did not differ in the extent to which they participated in the 24-month assessment phase; however, compared to the TD group, proportionally fewer FHx children participated in this assessment phase (p=0.03). Similarly, when individual measures were examined (Table 9), there were no significant group differences in the proportion of ASz and TD children who provided psychosocial stress, salivary cortisol, or pituitary volume data (p>0.45). However, relative to the TD group, FHx children were significantly less likely to have provided salivary cortisol data (p=0.05). This difference likely reflects the fact that recruitment of FHx children via medical records did not commence until after school screening was underway: as such, some FHx children had not completed the 24-month assessment phase at the time of analyses.

Within-group analyses were conducted to compare children who participated in the 24-month assessment phase and children who did not on psychopathology measures completed at screening (Table 10). Analyses were performed on continuous psychopathology variables rather than dichotomous variables owing to the small group sizes. These analyses confirmed that, across all three groups, children who participated in the 24-month follow-up phase did not differ significantly from children who did not on any of the four SDQ psychopathology scales or on total PLE scores at screening. However, analyses conducted in the ASz and TD groups should be interpreted with caution owing to the extremely small group sizes. Within-group analyses also indicated that participants who provided psychosocial stress, salivary cortisol, and pituitary volume data did not differ from those who did not provide these data on psychopathology scores at screening (p>0.09, data not shown).

Table 9. Longitudinal follow-up of the CHADS cohort and completion of measures examined in this thesis

	ASz (*n*=39)	FHx (*n*=32)	TD (*n*=46)	Statistics	
				ASz vs. TD	FHx vs. TD
Participated in the 24-month assessment phase; *n* (%)	35 (90)	25 (78)	44 (96)	*FE* p=0.41	***FE* p=0.03**
Provided psychosocial stress data; *n* (%)	34 (87)	24 (75)	42 (91)	*FE* p=0.73	*FE* p=0.06
Provided salivary cortisol data; *n* (%)	33 (85)	22 (69)	40 (87)	χ^2=0.10 p=0.76	**χ^2=3.84 p=0.05**
Provided pituitary gland volume data; *n* (%)	30 (77)	22 (69)	32 (70)	χ^2=0.58 p=0.45	χ^2=0.00 p=0.94

Note. Groups are not mutually exclusive - six cases met both ASz and FHx criteria and are included in both groups. *FE*: Fisher's exact.

Table 10. Participation in the 24-month follow-up phase and psychopathology at screening by group

	ASz (*n*=39)			FHx (*n*=32)			TD (*n*=46)		
	No FU (*n*=4)	FU (*n*=35)	Statistics	No FU (*n*=7)	FU (*n*=25)	Statistics	No FU (*n*=2)	FU (*n*=44)	Statistics
SDQ Emotional symptoms; mean ± SE	6.3 ± 1.7	4.4 ± 0.4	p=0.29	3.0 ± 1.2	2.8 ± 0.5	p=0.86	3.5 ± 0.5	2.5 ± 0.3	p=0.50
SDQ Conduct problems; mean ± SE	1.5 ± 0.6	2.9 ± 0.4	p=0.25	1.4 ± 0.7	2.8 ± 0.5	p=0.18	0.5 ± 0.5	1.0 ± 0.2	p=0.73
SDQ Hyperactivity/inattention; mean ± SE	3.3 ± 0.5	6.1 ± 0.5	p=0.07	3.6 ± 0.9	4.0 ± 0.5	p=0.72	2.5 ± 0.5	2.1 ± 0.3	p=0.91
SDQ Peer relationship problems; mean ± SE	4.3 ± 0.6	3.1 ± 0.4	p=0.38	1.4 ± 0.4	1.8 ± 0.3	p=0.76	2.0 ± 0.0	0.8 ± 0.2	p=0.14
Psychotic-like experiences; mean ± SE	9.8 ± 2.1	8.0 ± 0.5	p=0.33	5.6 ± 1.7	4.4 ± 0.8	p=0.48	2.5 ± 0.5	1.7 ± 0.2	p=0.41

Note. No FU: Not participated in the 24-month assessment phase; FU: participated in the 24-month assessment phase. Statistical analyses: Mann-Whitney U; analyses conducted in ASz and TD groups should be interpreted with caution due to extremely small group sizes.

3.5 Measures

The primary measures examined in this thesis, namely, psychosocial stress, salivary cortisol, pituitary volume, and neurocognitive function, are described in their respective chapters. Details of the methods used to assess sociodemographic characteristics and current psychopathology are provided in the following section.

3.5.1 Sociodemographic characteristics

Ethnicity

Participant ethnicity was determined using detailed information obtained from caregivers during the FIGS (Maxwell, 1992), a semi-structured interview designed to elicit information regarding mental health problems among first-, second-, and third-degree relatives. At the start of each interview, caregivers completed a detailed family tree, and, for each family member, provided information on their ethnicity and country of birth. These data were used to assign an ethnic group to each participant; groups were subsequently collapsed into white British, white other, black (i.e., black African and black Caribbean), and other.

Socioeconomic status

Caregivers completed a semi-structured interview to provide information on employment status, current occupation (or previous occupation for those not currently employed), and employer establishment size. Data were then coded according the UK National Statistics Socio-economic Classification (Office for National Statistics, 2010), and used to derive three classes: (i) higher managerial, administrative, and professional occupations, (ii) intermediate occupations, and (iii) routine and manual occupations. Relevant data were obtained for all caregivers residing with the child. Where two household representatives were identified, the 'dominance' approach was used to allocate a socioeconomic class to the family unit based on the caregiver with the highest socioeconomic status (Rose & Pevalin, 2003).

3.5.2 Psychopathology

Internalising and externalising symptoms

At the 24-month assessment phase, caregivers and children completed the Child Behaviour Checklist for school-aged children [CBCL (Achenbach & Rescorla, 2001)] and the accompanying Youth Self-Report (YSR), respectively. The CBCL/YSR is one of the most widely-used measures of childhood psychopathology; the measure has been evaluated extensively in child and adolescent populations and exhibits high reliability and validity (Achenbach & Rescorla, 2001). The CBCL and YSR incorporate a checklist of problems occurring during the past six months (CBCL: 113 items; YSR: 112 items); each item is scored on a three-point scale (0 'not true', 1 'somewhat true or sometimes true', or 2 'very true or often true'). The problem checklists assess eight empirically derived syndrome subscales. The current study examined caregiver- and child-reported scores on the internalising scale (sum of anxious-depressed, withdrawn-depressed, and somatic complaints subscales) and the externalising scale (sum of rule breaking and aggressive behaviour subscales).

Psychotic-like experiences

At the 24-month follow-up assessment, children re-completed the nine-item PLE measure included in the antecedent screening questionnaire (Laurens et al., 2007; Laurens et al., 2012) to provide ratings of current experiences. This measure (described in Section 3.2.1) included a range of hallucination- and delusion-like experiences, each rated on a three-point scale (0 'not true', 1 'somewhat true', or 2 'certainly true'). Scores on each item were summed to provide a total PLE score (total score range: 0-18).

3.6 Analysis strategy

3.6.1 Examination of group differences

As described previously, children meeting both ASz and FHx criteria were retained in both groups in order to most accurately reflect the ASz and FHx populations from which these participants were sampled. As these groups were not therefore mutually exclusive, the effect of ASz and FHx status on psychosocial stress, salivary cortisol, and pituitary volume was tested in independent analyses. Thus, ASz and FHx groups were each examined relative to the TD group but were not directly compared to each other.

3.6.2 Regression analyses

Linear regression analyses were used to examine the effect of risk status (ASz vs. TD and FHx vs. TD) on continuous outcome measures (number of life events, previous and current distress relating to negative life events, daily hassles frequency scores, daily hassles distress scores, cortisol awakening response, diurnal cortisol levels, and pituitary volume) and logistic regression analyses were employed for binary outcome measures (exposure to physical punishment). The distributions of all continuous outcome measures were examined prior to undertaking linear regression analyses using both visual inspection techniques (histograms) and statistical methods (skewness and kurtosis statistics) in order to check that these variables were approximately normally distributed.

3.6.3 Effect size computations

For all primary measures, standardised mean differences (*d*) were computed to indicate the magnitude of differences between each risk group (ASz and FHx) and the TD group. These effect sizes were computed to facilitate comparisons across the at-risk groups (which were not directly compared to one another) and across measures

(i.e., psychosocial stress, salivary cortisol, and pituitary volume). Whilst *d* is usually calculated from means and standard deviations (i.e., the mean difference divided by the pooled standard deviation) established methods exist for deriving *d* from unstandardised regression coefficients (Lipsey & Wilson, 2001), allowing that the magnitude of group differences can be examined after adjusting for additional variables. Specifically, the unstandardised regression coefficient (*B*) for the variable 'group' (where TD=0 and ASz/FHx=1) is equal to the difference between the two group means. Thus, as shown in the formulas below, *B* divided by the pooled standard deviation of the dependent variable produces an estimate of *d*. For binary measures, odds ratios were converted into *d* using the formula provided below (Lipsey & Wilson, 2001). As defined by Cohen (1992), for effect sizes derived from standardised mean differences, values of 0.20, 0.50, and 0.80 corresponded to 'small', 'medium', and 'large' effects, respectively.

Equation 1. Computation of *d* from unstandardised regression coefficients

$$\boldsymbol{d = \left(\frac{\overline{X}_1 - \overline{X}_2}{pooled\ SD}\right)} \quad \textbf{AND} \quad \boldsymbol{B = \overline{X}_1 - \overline{X}_2} \quad \textbf{THUS} \quad \boldsymbol{d = \left(\frac{B}{pooled\ SD}\right)}$$

Note. $\overline{X}$: Group mean; *SD*: standard deviation; *B*: unstandardised regression coefficient.

Equation 2. Computation of *d* from odds ratios

$$\boldsymbol{d = \frac{ln\ (OR)}{\left(\frac{\pi}{\sqrt{3}}\right)}}$$

Note. *ln*: Natural logarithm; *OR*: odds ratio.

3.6.4 Stratified analyses

As described in Chapter 2, an additional aim of this thesis was to examine the extent to which demographic factors (e.g., age, puberty, sex, socioeconomic status, ethnicity, and substance use) influence the relationship between risk status and psychosocial stress susceptibility/HPA axis function. All regression analyses were therefore adjusted for demographic factors that differed significantly between the groups or that were associated with the primary measures. Where adjustment for demographic factors led to a substantial change in the effect size associated with risk status – as denoted by a change in $d > 0.30$ (which represents the numerical difference between 'small', 'medium', and 'large' effects) – stratified analyses were performed to further explore the effect of these variables.

3.6.5 Correlation analyses

Within-group correlation analyses were conducted to examine relationships between the primary measures (experiences of psychosocial stress, salivary cortisol, and pituitary volume) and current psychopathology. In Chapter 7, correlations between neurocognitive function and psychosocial stress and salivary cortisol were also preformed. Prior to undertaking analyses, all variables were examined to ensure that they were approximately normally distributed. Pearson's '*r*' correlation analyses were used for normally distributed variables and Spearman's rho '*ρ*' correlation analyses were employed for non-normally distributed variables. Correlation coefficients of 0.10, 0.30, and 0.50 constituted 'small', 'medium', and 'large' effect sizes, respectively (Cohen, 1992).

CHAPTER 4 Psychosocial stress in children at elevated risk for schizophrenia

4.1 Introduction

The diathesis-stress model of schizophrenia proposes that exposure to psychosocial stressors can trigger the onset of psychosis among individuals with an underlying vulnerability for the disorder (Rosenthal, 1970; Zubin & Spring, 1977). Indeed, retrospective studies of patients with schizophrenia and prospective population cohort studies indicate that psychosocial stressors (encompassing major life events, childhood trauma, and milder daily hassles) contribute to the development and maintenance of psychosis (Myin-Germeys & van Os, 2007; Phillips et al., 2007; Varese et al., 2012). Furthermore, stressful events have been found to elicit greater emotional reactivity in individuals with psychosis compared to healthy controls (Myin-Germeys et al., 2001). Whilst these studies implicate stress in the aetiology of schizophrenia, the study of individuals at elevated risk for the disorder provides the opportunity to directly test whether schizophrenia vulnerability is associated with increased susceptibility to psychosocial stress (i.e., increased exposure to psychosocial stressors and greater reactivity to these exposures).

There is evidence that individuals identified as being at elevated risk for schizophrenia on account of their clinical presentation experience greater exposure to psychosocial stressors. Relative to healthy youth, higher levels of trauma have been observed among UHR youth and children reporting PLEs (Kelleher et al., 2008; Addington et al., 2013; Kelleher et al., 2013c; Sahin et al., 2013; Tikka et al., 2013), and adolescents with SPD and those reporting PLEs have been found to experience a greater number of recent life events (De Loore et al., 2007; Tessner et al., 2011). It is unclear whether this is also true of individuals at elevated risk due to a family history

of psychosis, and no study has yet compared individuals with different vulnerability profiles (i.e., symptomatic-risk vs. genetic-risk). However, both UHR youth (Palmier-Claus et al., 2012) and adults with a family history of psychosis (Myin-Germeys et al., 2001) have been found to show greater emotional reactivity to stressful experiences than healthy controls. Similarly, studies of adolescents with SPD (Tessner et al., 2011) and UHR youth (Phillips et al., 2012) indicate that these youth are more distressed by daily hassles than their healthy peers. Whilst several of these studies have reported that psychosocial stress exposure is associated with psychotic symptoms and depression (Miller et al., 2001; Thompson et al., 2007; Tessner et al., 2011), whether psychosocial stress is more strongly related to psychopathology among high-risk individuals compared to healthy controls has yet to be established.

Chapter aims

This chapter aimed to examine exposure to psychosocial stressors and reactivity to these exposures in children with different vulnerability profiles for schizophrenia: (i) children presenting multiple antecedents of schizophrenia (ASz), (ii) children with a family history of illness (FHx), and (iii) typically-developing (TD) children. A further aim was to determine whether the relationship between psychosocial stress and current psychopathology is associated with risk status.

Hypotheses

1a. ASz and FHx children will be exposed to higher levels of psychosocial stressors compared to the TD group.

1b. Relative to TD children, both ASz and FHx children will be more distressed by psychosocial stressors.

1c. Experiences of psychosocial stress will be more strongly associated with current psychopathology among ASz and FHx children than in TD children.

4.2 Methods

4.2.1 Participants and procedure

Chapter 3 provides full details of the recruitment procedure. In brief, ASz and TD children were recruited using a novel, school-based, community-screening method (Laurens et al., 2007; Laurens et al., 2011). FHx children were identified either via the caregiver screening questionnaire, which included items assessing family mental health difficulties, or as relatives of individuals with schizophrenia or schizoaffective disorder who were identified via medical records. Children eligible for the ASz, FHx, and TD groups were invited to participate in a longitudinal study of child development. This chapter primarily examines psychosocial stress data collected at the 24-month assessment phase.

4.2.2 Negative life events

Participants completed an eight-item self-report measure which assessed exposure to a range of child-appropriate negative life events (Heubeck & O'Sullivan, 1998), including: (i) the death of someone close, (ii) parental separation or divorce, (iii) experiencing a fire or natural disaster, (iv) being involved in a serious car accident, (v) serious illness requiring hospitalisation, (vi) serious illness in the parent, (vii) being the victim of burglary, and (viii) being the victim of any crime. For each event, participants were asked whether it had ever happened to them (yes or no), how distressed they had felt at the time of the event, and how much the event distressed them currently. Both distress ratings were scored on a four-point scale (0 'not at all', 1 'a little', 2 'somewhat', and 3 'a lot'). The number of negative life events was summed to give a total score. Distress ratings for each item were summed and divided by the number of items endorsed to provide two average distress scores (previous and current).

4.2.3 Daily hassles

Children completed a 37-item questionnaire, adapted from Heubeck and O'Sullivan (1998) assessing school-related daily hassles in four domains: scholastic (9 items: e.g., 'I have to sit a test' and 'my homework is too hard'), home (4 items: e.g., 'my parents want me to do better' and 'my parents are critical of my homework'), peer (16 items: e.g., 'other children make fun of me, tease me, or pick on me' and 'I feel left out'), and teacher (8 items: e.g., 'a teacher does not listen to me' and 'a teacher is unfair to me or other children'). For each item, participants indicated on a four-point scale how frequently the hassle had occurred during the past six months (0 'never', 1 'rarely', 2 'sometimes', and 3 'often'), and how distressed this event made them feel (0 'not at all', 1 'a little', 2 'somewhat', and 3 'a lot'). Frequency ratings for items included in each subscale were summed to give total frequency scores for each of the four domains (reflecting the number of hassles experienced and how often they occurred). A total daily hassles frequency score was computed by summing frequency scores on all 37 items. Average domain distress scores were created by summing distress ratings on contributing items and dividing this score by the number of endorsed items (reflecting the average distress per item); the same procedure was performed with all items to obtain an overall average daily hassle distress score. After excluding one item from the scholastic domain which was poorly correlated with the remaining items ('I have trouble with reading, writing, or spelling'), Cronbach's alpha coefficients were moderate-to-high (range: 0.71-0.89) for all domain frequency and distress scales except the home frequency scale (α=0.55) and the home distress scale (α=0.59), which likely reflects the few items contributing to the home domain. Both scales were retained in analyses but should be interpreted with caution. Internal consistencies for the total frequency and overall distress scales were high (0.88 and 0.92, respectively; note, both scales included the item excluded from the scholastic domain).

4.2.4 Physical punishment

Exposure to physical punishment was assessed using the corporal punishment scale of the Alabama Parenting Questionnaire [APQ (Shelton et al., 1996)], completed by the child and their caregiver independently. The APQ is a widely-used measure of parenting practices which demonstrates high reliability and validity (Shelton et al., 1996; Dadds et al., 2003). The corporal punishment scale includes three items: (i) spanking, (ii) slapping, and (iii) hitting with a belt, switch, or other object; children and their caregivers were asked to indicate how often each item typically occurred in their home on a five-point scale (1 'never', 2 'almost never', 3 'sometimes', 4 'often', and 5 'always'). Unlike the negative life event and daily hassles measures, the APQ was administered at both the baseline and 24-month assessment phases (rather than at the 24-month assessment only). Thus, in order to provide a more comprehensive index of exposure to physical punishment occurring during the time interval between initial identification of participants and the 24-month assessment phase, both baseline and 24-month assessment data were examined in this chapter. Internal consistencies for the child- and caregiver-report corporal punishment scales were moderate-to-high at the baseline assessment (α=0.85 and 0.65, respectively) and 24-month assessment (α=0.90 and 0.72, respectively). A dichotomous summary variable for physical punishment incorporating reports from both informants and both assessment phases was created, defined as a child- or caregiver-reported score of three or more on at least one of the three items at the baseline and/or 24-month assessment. To assess more severe forms of physical punishment, a further dichotomous variable was derived, defined as a child- or caregiver-reported score of three or more on the 'hitting with a belt, switch, or other object' item at the baseline and/or 24-month assessment.

4.2.5 Current psychopathology

Psychopathology measures were described in Chapter 3. In brief, participants and their caregivers completed the Child Behaviour Checklist [CBCL (Achenbach & Rescorla, 2001)] and the corresponding Youth Self-Report (YSR), respectively. The current study examines scores on the internalising and externalising scales of the CBCL/YSR. Children also re-completed the nine-item PLE measure (Laurens et al., 2012) assessing delusion- and hallucination-like experiences. Scores on each item were summed to provide a total PLE score (range: 0-18).

4.2.6 Statistical analyses

Independent samples t-tests, Mann-Whitney U tests, chi-squared tests, and Fisher's exact tests were used to examine group differences on demographic variables, APQ physical punishment scale scores, and current psychopathology. Independent samples t-tests, one way ANOVA's, correlation analyses, and chi-squared tests were used to explore associations between demographic variables and negative life events, daily hassles, and physical punishment in the total sample.

The effect of risk status on negative life event and daily hassles variables was examined using linear regression analyses. All daily hassles scales (frequency and distress) were approximately normally distributed, as was the number of negative life events (skewness and kurtosis values ≤ 1.00). However, the two negative life event distress scales (distress at the time of the event and current distress) showed minor departures from normality (skewness: 0.13 and 1.11; kurtosis: -1.36 and 0.57, respectively). Non-parametric analyses were performed on these two variables using quantile (median) regression in Stata version 12 which yielded an identical pattern of results to those obtained in linear regression analyses. Thus, in order to maintain consistency in the analytic method and software package employed, and to provide estimates of effect size which cannot be derived from the quantile regression

coefficients, parametric linear regression analyses were employed for all variables. In addition, logistic regression was used to determine the effect of risk status on physical punishment. In each regression analysis, the predictive effect of each risk group was tested independently (i.e., ASz and FHx children were examined relative to TD children but were not directly compared to each other due to several children meeting inclusion criteria for both groups). All regression analyses were subsequently adjusted for demographic factors that differed significantly between the groups or that were associated with psychosocial stress measures in preliminary analyses. Unstandardised regression coefficients from both unadjusted and adjusted analyses were used to derive standardised mean differences (*d*) as indices of effect size (Lipsey & Wilson, 2001). Odds ratios from logistic regression analyses were also converted into standardised effect sizes (Lipsey & Wilson, 2001). As described in Chapter 3, where adjustment for demographic factors led to a substantial change in the effect size associated with risk status (change in $d > 0.30$), stratified analyses were performed to further explore the effect of these variables.

Within-group correlation analyses were conducted to examine relationships between psychosocial stress variables and current psychopathology. Pearson's '*r*' correlation analyses were used for normally distributed variables (daily hassles scales, number of negative life events, and YSR scores) and Spearman's rho '*ρ*' correlation analyses for non-normally distributed variables (negative life event distress scales, APQ physical punishment scale scores, and CBCL and PLE scores). To limit the number of statistical tests conducted (and hence prevent inflation of type 1 error rate), only total scores on the daily hassles frequency and distress scales (as opposed to the four domain scores) were examined in the correlation analyses. All final analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 20.

Table 11. Sociodemographic characteristics of participants providing psychosocial stress data

	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Statistics	
				ASz vs. TD	FHx vs. TD
Age (years); mean ± SE	12.9 ± 0.2	13.2 ± 0.2	13.1 ± 0.2	*t*=1.00 *p*=0.32	*t*=-0.57 *p*=0.57
Time lapse: screening to stress assessment (years); mean ± SE	2.6 ± 0.1	2.6 ± 0.2	2.8 ± 0.1	*t*=0.86 *p*=0.39	*t*=0.77 *p*=0.44
Sex (male); *n* (%)	24 (71)	11 (46)	20 (48)	**χ^2=4.07 *p*=0.04**	χ^2=0.02 *p*=0.89
Ethnicity; *n* (%)				***FE*=8.72 *p*=0.03**	**χ^2=17.30 *p*=0.001**
White British	7 (21)	3 (13)	19 (45)		
White other	8 (23)	2 (8)	12 (29)		
Black	5 (15)	9 (37)	5 (12)		
Other	14 (41)	10 (42)	6 (14)		
Socioeconomic status; *n* (%) [a]				***FE*=15.29 *p*<0.001**	***FE*=14.22 *p*<0.001**
Higher managerial, administrative, and professional	14 (41)	12 (50)	35 (83)		
Intermediate	12 (35)	3 (13)	6 (14)		
Routine and manual	8 (24)	9 (37)	1 (3)		

Note. Five ASz+FHx cases are included in both groups. *FE*: Fisher's exact. [a] Socioeconomic status based on caregiver occupation.

4.3 Results

4.3.1 Sample characteristics

In total, 95 children completed assessments of psychosocial stress; 29 children met ASz criteria only, 19 met FHx criteria only, 5 met both ASz and FHx criteria, and 42 met TD criteria. The five ASz+FHx cases were included in both the ASz and FHx groups, yielding data for 34 ASz and 24 FHx children in total. As described previously, ASz and FHx groups were examined relative to the TD group only and not directly to each other. Sample characteristics are presented by group in Table 11. There were no significant group differences (ASz vs. TD or FHx vs. TD) in age or lapse of time between screening and assessment (p>0.30). ASz children were significantly more likely to be male compared to the TD group (p=0.04). When ASz and FHx groups were compared to the TD group, they each differed significantly on ethnicity (p<0.05) and socioeconomic status (p≤0.001).

4.3.2 Factors associated with experiences of psychosocial stress

Preliminary analyses were conducted to identify sociodemographic correlates of psychosocial stress. Age was negatively correlated with previous (ρ=-0.25, p=0.01) and current distress (ρ=-0.21, p=0.04) relating to negative life events. Additionally, ethnicity was significantly associated with home hassles frequency (F[3, 91]=5.35, p=0.002) and distress scores (F[3, 91]=2.80, p=0.05), and with physical punishment (χ^2=20.03, p<0.001). Specifically, children of black and 'other' ethnicity had higher scores on the home hassles frequency and distress scales and were more likely to experience physical punishment relative to white British and other white children. Socioeconomic status was also significantly associated with physical punishment (χ^2=24.10, p<0.001); children in the 'intermediate' and 'routine and manual' classes were more likely to experience physical punishment than those in the 'higher managerial, administrative, and professional' class.

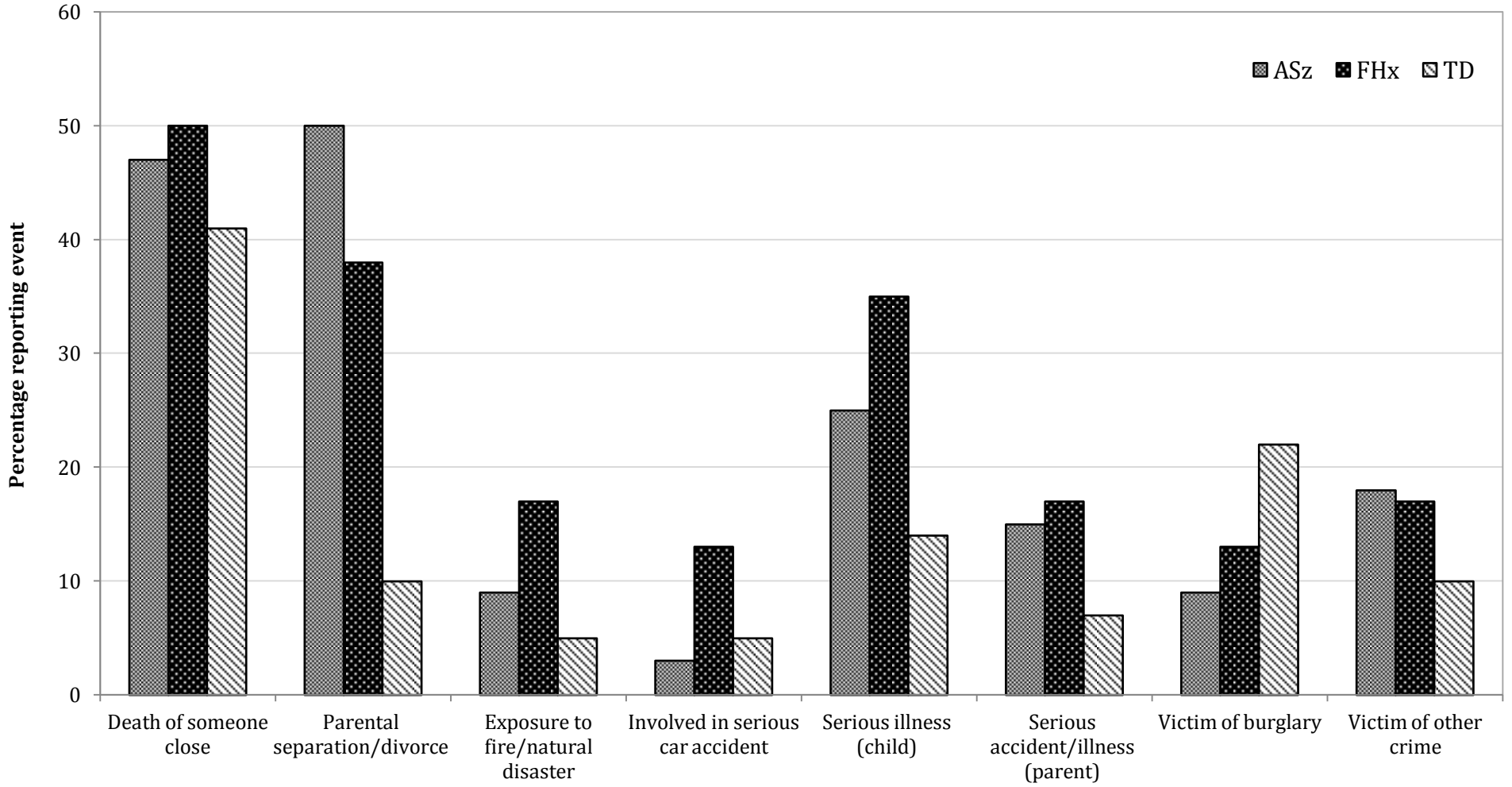

Figure 6. Prevalence of individual negative life events by group

4.3.3 Group differences in negative life events

Across the total sample, 73 children (77%) reported that they had experienced at least one negative life event; the most commonly-reported event being the death of someone close. Visual inspection of these data (Figure 6), indicated that the prevalence of each life event was higher among ASz and FHx children compared to the TD group with the exception that being involved a serious car accident was more common in the TD group than in the ASz group, and that TD children were more likely than both ASz and FHx children to have been the victim of burglary.

The mean number of negative life events experienced by the ASz, FHx, and TD groups was 1.74, 1.96, and 1.10, respectively (Table 12). Linear regression analyses indicated that FHx children reported a significantly higher number of negative life events than TD children, equating to a moderate effect size (d=0.66, p=0.01). This effect was not substantially changed after adjustment for demographic factors. Post-hoc tests confirmed that among FHx children, those with a first-degree relative with schizophrenia (n=8), and those with second-degree relative with the disorder (n=16), did not differ on the mean number of life events experienced (2.00 vs. 1.93, respectively; t=-0.09, p=0.93). In unadjusted analyses, FHx and TD children did not differ significantly on either of the negative life event distress scales (previous or current). After adjustment for demographic factors, the effect of FHx status on current distress was increased in magnitude and achieved statistical significance (d=0.70, p=0.04); stratified analyses were not employed as the magnitude of change in the effect size was just less than 0.30. The ASz group also reported a significantly greater number of negative life events relative to the TD group (d=0.55, p=0.02); this effect was largely unchanged after adjustment for demographic factors although estimates were less precise (d=0.48, p=0.09). ASz and TD children did not differ on the degree of distress experienced either at the time of the event or currently.

Table 12. Linear regression analyses examining the effect of risk status on negative life event exposure and distress

	Descriptive statistics			Statistical analyses								
	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Model [a]	ASz vs. TD				FHx vs. TD			
					d	*B*	(95% CI)	*p*	*d*	*B*	(95% CI)	*p*
Number of negative life events; mean ± SE	1.74 ± 0.20	1.96 ± 0.31	1.10 ± 0.19	Unadjusted	**0.55**	**0.64**	**(0.10 – 1.18)**	**0.02**	**0.66**	**0.86**	**(0.19 – 1.54)**	**0.01**
				Adjusted	0.48	0.57	(-0.09 – 1.22)	0.09	**0.77**	**0.99**	**(0.14 – 1.84)**	**0.02**
Distress at the time of the event; mean ± SE	1.44 ± 0.17	1.51 ± 0.23	1.26 ± 0.18	Unadjusted	0.16	0.18	(-0.33 – 0.69)	0.49	0.22	0.25	(-0.34 – 0.85)	0.39
				Adjusted*	-0.03	-0.04	(-0.63 – 0.55)	0.90	0.42	0.48	(-0.25 – 1.21)	0.19
Current distress relating to event; mean ± SE	0.88 ± 0.15	0.91 ± 0.19	0.58 ± 0.11	Unadjusted	0.37	0.30	(-0.07 – 0.66)	0.12	0.41	0.33	(-0.08 – 0.75)	0.12
				Adjusted*	0.16	0.13	(-0.31 – 0.57)	0.56	**0.70**	**0.54**	**(0.04 – 1.05)**	**0.04**

Note. Five ASz+FHx cases are included in both groups; *d*: standardised effect size; *B*: unstandardised regression coefficient. [a] Linear regression analyses without adjustment for potential confounders (unadjusted) and adjusted for sex, ethnicity, and socioeconomic status (adjusted), with age included as an additional covariate (adjusted*).

4.3.4 Group differences in daily hassles

Mean daily hassles frequency scores, reflecting the number of daily hassles experienced during the past six months and how often they were experienced, are presented by group in Table 13. Linear regression analyses were conducted to examine the effect of risk status on domain frequency scores. Relative to the TD group, ASz children were exposed to a higher frequency of peer-related (d=0.84, p=0.001) and teacher-related hassles (d=0.90, p<0.001). The effect of ASz status on hassles frequency scores in these domains remained moderate-to-large and statistically significant after adjustment for sex, ethnicity, and socioeconomic status (peer: d=1.02, p=0.001; teacher: d=0.73, p=0.008). In contrast, FHx children reported that they were more frequently exposed to home-related daily hassles than TD children (d=0.53, p=0.05). The effect size was reduced after adjusting for demographic factors (d=0.24, p=0.42), again, stratified analyses were not employed as the magnitude of change in the effect size was just less than 0.30.

Mean daily hassles distress scores for each domain are presented by group in Table 14; these scores reflect the average level of distress experienced per item. Linear regression analyses indicated that relative to the TD group, ASz children reported significantly greater distress regarding daily hassles across all four domains (p<0.03), these differences were moderate in magnitude (d range: 0.53–0.63). Adjustment for sex, ethnicity, and socioeconomic status, led to a moderate change in these effect sizes (magnitude of change in d<0.30 for all effects) and subsequently a significant effect of ASz status was observed for the scholastic-related, peer-related, and teacher-related distress scales (d range: 0.72–0.91, p<0.05). In contrast, relative to TD children, the FHx group reported that they were more distressed by hassles in the home domain only (d=0.53, p=0.05); a slight reduction in effect was observed after adjustment for demographic factors (d=0.42, p=0.21).

Table 13. Linear regression analyses examining the effect of risk status on frequency of daily hassles

	Descriptive statistics			Statistical analyses								
	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Model [a]	ASz vs. TD				FHx vs. TD			
					d	*B*	(95% CI)	*p*	*d*	*B*	(95% CI)	*p*
Scholastic hassles frequency; mean ± SE	12.09 ± 0.58	12.04 ± 0.84	11.00 ± 0.59	Unadjusted	0.30	1.09	(-0.59 – 2.76)	0.20	0.27	1.04	(-0.98 – 3.06)	0.31
				Adjusted	0.42	1.49	(-0.46 – 3.44)	0.13	0.23	0.85	(-1.50 – 3.19)	0.47
Home hassles frequency; mean ± SE	4.76 ± 0.41	4.92 ± 0.43	3.76 ± 0.35	Unadjusted	0.44	1.00	(-0.07 – 2.07)	0.07	**0.53**	**1.16**	**(0.02 – 2.29)**	**0.05**
				Adjusted	0.17	0.41	(-0.77 – 1.58)	0.49	0.24	0.53	(-0.78 – 1.85)	0.42
Peer hassles frequency; mean ± SE	14.94 ± 1.21	10.88 ± 1.37	9.31 ± 1.02	Unadjusted	**0.84**	**5.63**	**(2.51 – 8.76)**	**0.001**	0.24	1.57	(-1.82 – 4.96)	0.36
				Adjusted	**1.02**	**6.60**	**(2.81 – 10.40)**	**0.001**	0.47	3.00	(-1.22 – 7.23)	0.16
Teacher hassles frequency; mean ± SE	10.29 ± 0.77	8.29 ± 0.83	6.86 ± 0.52	Unadjusted	**0.90**	**3.44**	**(1.65 – 5.23)**	**<0.001**	0.40	1.44	(-0.41 – 3.28)	0.13
				Adjusted	**0.73**	**2.88**	**(0.78 – 4.98)**	**0.008**	0.30	1.09	(-1.20 – 3.37)	0.35

Note. Five ASz+FHx cases are included in both groups. *d*: standardised effect size; *B*: unstandardised regression coefficient. [a] Linear regression analyses without adjustment for potential confounders (unadjusted) and adjusted for sex, ethnicity, and socioeconomic status (adjusted).

Table 14. Linear regression analyses examining the effect of risk status on distress relating to daily hassles

	Descriptive statistics			Statistical analyses								
	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Model [a]	ASz vs. TD				FHx vs. TD			
					d	*B*	(95% CI)	*p*	*d*	*B*	(95% CI)	*p*
Scholastic hassles distress; mean ± SE	1.43 ± 0.08	1.17 ± 0.12	1.07 ± 0.10	Unadjusted	**0.63**	**0.37**	**(0.10 – 0.64)**	**0.008**	0.16	0.10	(-0.22 – 0.42)	0.54
				Adjusted	**0.91**	**0.50**	**(0.21 – 0.79)**	**0.001**	-0.01	-0.01	(-0.41 – 0.39)	0.97
Home hassles distress; mean ± SE	1.10 ± 0.10	1.11 ± 0.14	0.76 ± 0.10	Unadjusted	**0.53**	**0.34**	**(0.04 – 0.64)**	**0.03**	**0.53**	**0.35**	**(0.00 – 0.69)**	**0.05**
				Adjusted	0.33	0.21	(-0.14 – 0.56)	0.23	0.42	0.28	(-0.16 – 0.72)	0.21
Peer hassles distress; mean ± SE	1.15 ± 0.12	0.80 ± 0.14	0.81 ± 0.09	Unadjusted	**0.56**	**0.34**	**(0.06 – 0.63)**	**0.02**	0.00	-0.00	(-0.32 – 0.32)	0.99
				Adjusted	**0.85**	**0.50**	**(0.17 – 0.82)**	**0.003**	0.13	0.08	(-0.32 – 0.48)	0.70
Teacher hassles distress; mean ± SE	1.23 ± 0.10	0.84 ± 0.13	0.91 ± 0.09	Unadjusted	**0.58**	**0.33**	**(0.06 – 0.59)**	**0.02**	-0.12	-0.07	(-0.38 – 0.24)	0.64
				Adjusted	**0.72**	**0.40**	**(0.09 – 0.70)**	**0.01**	-0.19	-0.11	(-0.51 – 0.28)	0.57

Note. Five ASz+FHx cases are included in both groups. *d*: standardised effect size; *B*: unstandardised regression coefficient. [a] Linear regression analyses without adjustment for potential confounders (unadjusted) and adjusted for sex, ethnicity, and socioeconomic status (adjusted).

4.3.5 Group differences in physical punishment

Mean scores on the APQ physical punishment scales (child- and caregiver-report) are presented by group in Table 15. Relative to the TD group, both ASz and FHx children obtained significantly higher scores on the child-report physical punishment scales at both the baseline and 24-month assessment ($p \leq 0.001$), in addition, ASz children were characterised by significantly higher scores on the caregiver-report physical punishment scale at the 24-month assessment ($p=0.01$).

Dichotomous measures of physical punishment were created using the procedures described previously in order to incorporate information from both informants (i.e., child and caregiver) and both assessment phases (i.e., baseline and 24-month). As shown in Table 16, the prevalence of physical punishment among ASz, FHx, and TD children was 47%, 54%, and 24%, respectively. Severe physical punishment (i.e., hitting with a belt or other object) was experienced by 9 (27%) ASz children and 9 (38%) FHx children; however, none of the TD group had experienced these more severe forms of punishment. Thus, logistic regression analyses examining the effect of risk status on physical punishment were subsequently performed only on the more leniently defined variable. Relative to the TD group, the odds of being exposed to physical punishment were 2.84 times higher among ASz children ($p=0.04$), equating to a large effect size ($d=1.57$). Adjustment for sex, ethnicity, and socioeconomic status, led to a substantial reduction in the effect size associated with ASz status, such that no statistically significant effect of ASz status was observed ($d=0.61$, $p=0.89$). Similarly, unadjusted logistic regression analyses indicated that FHx children were 3.78 times more likely to experience physical punishment than TD children ($d=2.09$, $p=0.02$). Again, adjustment for demographic factors substantially reduced the effect size associated with FHx status ($d=0.70$, $p=0.77$).

Table 15. APQ physical punishment scale scores by group

	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Statistics	
				ASz vs. TD	FHx vs. TD
Baseline APQ physical punishment score (child-report); mean ± SE	6.03 ± 0.64	5.64 ± 0.68	3.56 ± 0.18	***U*=325.5 *p*<0.001**	***U*=242.0 *p*=0.001**
Baseline APQ physical punishment score (caregiver-report); mean ± SE	4.44 ± 0.29	4.48 ± 0.34	3.93 ± 0.20	*t*=-1.49 *p*=0.14	*t*=-1.48 *p*=0.14
24-month APQ physical punishment score (child-report); mean ± SE	4.53 ± 0.43	5.00 ± 0.58	3.26 ± 0.13	***U*=523.0 *p*=0.01**	***U*=295.0 *p*=0.001**
24-month APQ physical punishment score (caregiver-report); mean ± SE	4.26 ± 0.24	4.39 ± 0.40	3.76 ± 0.20	***U*=536.0 *p*=0.04**	*U*=382.5 *p*=0.11

Note. Five ASz+FHx cases are included in both groups. APQ: Alabama Parenting Questionnaire. Missing data: baseline – child (*n*=9); baseline – caregiver (*n*=2); 24-month – child (*n*=1); 24-month – caregiver (*n*=1).

Table 16. Logistic regression analyses examining the effect of risk status on exposure to physical punishment

	Descriptive statistics			Statistical analyses								
	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Model [a]	ASz vs. TD				FHx vs. TD			
					d	*OR*	(95% CI)	*p*	*d*	*OR*	(95% CI)	*p*
Physical punishment (ever experienced); *n* (%)	16 (47)	13 (54)	10 (24)	Unadjusted	**1.57**	**2.84**	**(1.07 – 7.57)**	**0.04**	**2.09**	**3.78**	**(1.30 – 11.05)**	**0.02**
				Adjusted	0.61	1.11	(0.27 – 4.54)	0.89	0.70	1.28	(0.25 – 6.44)	0.77

Note. Five ASz+FHx cases are included in both groups. *d*: standardised effect size; *OR*: odds ratio. [a] Logistic regression analyses without adjustment for potential confounders (unadjusted) and adjusted for sex, ethnicity, and socioeconomic status (adjusted).

Ethnicity and socioeconomic status were associated with physical punishment in preliminary analyses, and their inclusion in the regression models attenuated the effect of both ASz and FHx status; stratified analyses were therefore performed to explore the influence of these variables (Table 17). Ethnicity groups were combined to derive two summary groups: 'white' ('white British' and 'white other') and 'other' ('black' and 'other'). A similar procedure was performed for the socioeconomic classes whereby the 'higher managerial, administrative, and professional' group was retained and the 'intermediate' and 'routine and manual' groups were combined, yielding a 'higher' and 'lower' group. Stratified analyses indicated that the effect of risk status was indeed influenced by ethnicity and socioeconomic status. Specifically, ASz and FHx children were more likely to experience physical punishment than TD children when 'white' children were examined (effects were moderate-to-large but did not achieve statistical significance), but this pattern was not present among children of 'other' ethnicity. Similarly, among children of 'higher' socioeconomic status, physical punishment was more common among ASz and FHx children than in TD children (equating to moderate-to-large, although not statistically significant, effects), yet in the 'lower' socioeconomic group the reverse pattern was observed.

Table 17. Stratified analyses examining risk status and physical punishment

	Physical punishment; *n* (%)				
	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Statistics *	
				ASz vs. TD	FHx vs. TD
Ethnicity					
White	4 (27)	2 (40)	3 (10)	*d*=0.67 *p*=0.19	*d*=1.01 *p*=0.13
Other	12 (63)	11 (58)	7 (64)	*d*=-0.01 *p*=1.00	*d*=-0.13 *p*=1.00
SES					
Higher	4 (29)	4 (33)	4 (11)	*d*=0.62 *p*=0.20	*d*=0.75 *p*=0.18
Lower	12 (60)	9 (75)	6 (86)	*d*=-0.76 *p*=0.36	*d*=-0.38 *p*=1.00

Note. Five ASz+FHx cases included in both groups.* Fisher's exact. SES: socioeconomic status; *d*: standardised effect size.

Table 18. Group differences in current psychopathology

	ASz (*n*=34)	FHx (*n*=24)	TD (*n*=42)	Statistics	
				ASz vs. TD	FHx vs. TD
YSR Internalising scale (child-report); mean ± SE	12.30 ± 1.39	10.04 ± 1.39	9.38 ± 1.11	*t*=-1.66 *p*=0.10	*t*=-0.36 *p*=0.72
YSR Externalising scale (child-report); mean ± SE	11.55 ± 1.12	9.04 ± 1.39	6.24 ± 0.73	***t*=-4.11 *p*<0.001**	***t*=-1.97 *p*=0.05**
CBCL Internalising scale (caregiver-report); mean ± SE	11.47 ± 1.43	8.04 ± 2.17	6.40 ± 0.80	***U*=445.0 *p*=0.005**	*U*=460.0 *p*=0.56
CBCL Externalising scale (caregiver-report); mean ± SE	12.26 ± 1.90	9.92 ± 2.39	4.74 ± 0.85	***U*=386.0 *p*=0.001**	*U*=401.5 *p*=0.17
Psychotic-like experiences (child-report); mean ± SE	2.15 ± 0.46	1.92 ± 0.53	0.85 ± 0.21	***U*=506.5 *p*=0.03**	*U*=389.0 *p*=0.12

Note. Five ASz+FHx cases are included in both groups. YSR: Youth Self-Report; CBCL: Child Behaviour Checklist. Missing data: YSR (*n*=2); psychotic-like experiences (*n*=1).

4.3.6 Psychosocial stress and current psychopathology

Psychopathology data are presented in Table 18. Relative to the TD group, ASz children obtained significantly higher scores on the CBCL internalising and externalising scales, the YSR externalising scale, and the PLE questionnaire (p<0.03), whilst FHx children were characterised by higher YSR externalising scores (p=0.05).

Parametric ('r') and non-parametric ('ρ') correlation analyses were conducted to examine associations between psychosocial stress measures and current psychopathology (Table 19). In the ASz group only, moderate correlations were observed between the total number of negative life events and PLEs (ρ=0.50, p=0.003). Total daily hassles frequency scores were positively correlated with PLEs across all three groups; strong correlations were observed among ASz (ρ=0.53, p=0.001) and FHx children (ρ=0.55, p=0.006), but a moderate correlation only was observed in the TD group (ρ=0.33, p=0.04). Among FHx children, total daily hassles frequency scores were also positively correlated with child-reported internalising (ρ=0.49, p=0.02) and externalising symptoms (ρ=0.48, p=0.02). Similarly, in the TD group, positive correlations were observed between total daily hassles frequency scores and child-reported internalising (r=0.63, p<0.001) and externalising symptoms (r=0.57, p<0.001), and total daily hassles distress scores were associated with child-reported internalising symptoms (r=0.67, p<0.001) and PLEs (ρ=0.41, p=0.002). Among FHx children, child-reported (ρ=0.48, p=0.03) and caregiver-reported (ρ=0.42, p=0.04) physical punishment scores at baseline were positively correlated with YSR internalising symptoms whilst child-reported physical punishment scale scores at the 24-month follow-up were positively correlated with PLEs (ρ=0.49, p=0.02). In addition, caregiver-reported physical punishment scale scores at the 24-month follow-up were associated with CBCL externalising symptoms in the TD group (ρ=0.46, p=0.002).

Table 19. Correlations between psychosocial stress measures and current psychopathology

	ASz (*n*=34)					FHx (*n*=24)					TD (*n*=42)				
	YSR-I	YSR-E	CBCL-I	CBCL-E	PLE	YSR-I	YSR-E	CBCL-I	CBCL-E	PLE	YSR-I	YSR-E	CBCL-I	CBCL-E	PLE
Negative life events															
Total number	0.12	0.26	-0.32	-0.07	0.50**	0.23	0.15	0.00	-0.12	0.31	0.05	-0.03	0.02	0.06	-0.10
Previous distress	0.03	-0.08	-0.06	-0.17	0.17	0.40	0.09	-0.12	-0.23	0.07	0.03	0.05	-0.10	0.10	-0.24
Current distress	0.10	0.11	-0.06	-0.28	0.23	0.39	0.12	0.10	-0.21	0.08	0.08	0.08	-0.11	0.12	-0.20
Daily hassles															
Total frequency score	0.28	0.26	-0.26	-0.14	0.53**	0.50*	0.47*	0.36	0.27	0.55**	0.63**	0.57**	-0.22	-0.05	0.33*
Average distress score	0.12	0.00	0.09	-0.13	0.26	0.07	-0.01	0.05	0.13	0.01	0.67**	0.24	-0.02	-0.04	0.41**
APQ physical punishment															
Baseline – child	0.07	-0.09	0.23	0.21	0.01	0.12	0.28	0.48*	0.33	-0.05	0.25	0.26	0.13	0.06	-0.02
Baseline – caregiver	-0.17	0.21	0.09	0.26	-0.09	-0.04	0.20	0.43*	0.26	0.04	-0.04	0.14	0.09	0.13	-0.09
24-month – child	0.16	0.28	-0.21	-0.08	0.22	0.23	0.33	0.10	-0.03	0.49*	0.17	0.03	0.06	0.02	-0.09
24-month – caregiver	-0.09	0.24	-0.02	0.31	0.17	-0.10	0.31	0.01	0.30	0.01	0.04	0.13	0.19	0.36*	0.01

Note. * $p<0.05$; ** $p<0.01$; YSR-I: Youth Self-Report – Internalising scale; YSR-E: Youth Self-Report – Externalising scale; CBCL-I: Child Behaviour Checklist – Internalising scale; CBCL-E: Child Behaviour Checklist – Externalising scale; PLE: psychotic-like experiences; APQ: Alabama Parenting Questionnaire. Missing data: YSR (*n*=2); PLE (*n*=1); APQ baseline – child (*n*=9) and caregiver (*n*=2); APQ 24-month – child (*n*=1) and caregiver (*n*=1).

4.4 Discussion

The aim of this chapter was to examine experiences of psychosocial stress in children with different vulnerability profiles for schizophrenia: those who present multiple antecedents of the disorder, and those with a family history of illness. Relative to TD children, both FHx and ASz children were more frequently exposed to major negative life events and milder daily hassles respectively, and reported greater distress resulting from these experiences. Physical punishment was also more common among ASz and FHx children compared to TD children, and stratified analyses indicated that ethnicity and socioeconomic status may influence this relationship. Correlation analyses demonstrated that the association between psychosocial stress and current psychopathology was associated with risk status.

4.4.1 Comparison with previous research

Negative life events

Relative to the TD group, FHx and ASz children were exposed to a greater number of negative life events. There was no substantial change in the magnitude of either effect after adjusting for demographic factors, although the effect of ASz status just missed statistical significance in adjusted analyses (likely to reflect a loss in statistical power). Studies examining life events in older samples of high-risk adolescents and young adults have yielded inconsistent findings. Miller and colleagues observed no difference in the number of major or minor life events reported by youth with a family history of schizophrenia and healthy controls (Miller et al., 2001). Furthermore, two recent studies reported that UHR youth (Phillips et al., 2012; Devylder et al., 2013) experienced fewer recent life events than healthy controls, although this was only statistically significant in the former study (Phillips et al., 2012). In contrast, adolescents with SPD (Tessner et al., 2011) and children reporting PLEs (De Loore et al., 2007) have been found to experience a greater number of life

events than their healthy peers. The inconsistency across studies may relate to differences in the age of participants and/or life event measures employed. Measures used in previous studies also assessed positive events, which may have masked any group differences in negative life events. Indeed, larger effect sizes were observed in the study of adolescents with SPD when undesirable life events were examined in isolation (Tessner et al., 2011). In addition, despite the fact that all previous studies (with the exception of Miller and colleagues) examined recent life events only, the mean number of events reported by participants in these studies was far higher than in the current study. This may reflect the younger age of the current sample (i.e., life events being less common in childhood than in later life), or it may be that previous studies examined more frequently-occurring events that are less severe. FHx children also reported that they were currently more distressed by negative life events than TD children, although this was not also observed among ASz children. Youth at UHR have also been found to be more distressed by negative life events than healthy controls (Phillips et al., 2012).

Daily hassles

ASz children were more frequently exposed to peer- and teacher-related daily hassles than TD children, and were more distressed by hassles across all domains; findings remained broadly consistent after adjustment for demographic factors. In contrast, FHx children obtained higher scores on the home hassles frequency and distress scales only; these effects were somewhat attenuated after adjustment for demographic factors. Two previous studies, one of youth at UHR (Phillips et al., 2012) and the other of adolescents with SPD (Tessner et al., 2011), did not observe a higher frequency of daily hassles in high-risk youth relative to healthy controls. However, both studies reported that high-risk youth were more distressed by these experiences. Again, differences in findings are likely to relate to the measures

employed. Previous studies examined hassles occurring during the past month (Phillips et al., 2012), or during the previous and current day (Tessner et al., 2011), whereas the current study assessed hassles experienced over the past six months. Furthermore, in contrast to previous studies, the measure of daily hassles employed in the current study indexed not only the number of hassles experienced but also how often they occurred, which may be a more sensitive measure.

Physical punishment

Relative to the TD group, both ASz and FHx children were significantly more likely to experience physical punishment (odds ratios: 2.84 and 3.78, respectively). In both groups, the effect of risk status was substantially reduced after adjusting for demographic factors. This is the first study to examine physical punishment in high-risk youth and healthy controls. However, a large epidemiological study found that individuals exposed to physical punishment were 2.46 times more likely to meet criteria for SPD (Afifi et al., 2012), and that physical punishment was more strongly associated with SPD than any other personality disorder. Additionally, the authors observed that the association between SPD and physical punishment was significant after adjustment for demographic factors, which may reflect the fact that, unlike the current study, indices of socioeconomic status were positively (and not negatively) associated with physical punishment. Physical punishment and childhood maltreatment are not separate entities but represent varying degrees of physical force which lie on a continuum of severity (Afifi et al., 2012). Thus, the current findings are consistent with previous studies showing that UHR youth are more likely to experience physical abuse than healthy controls (Addington et al., 2013; Sahin et al., 2013; Tikka et al., 2013). The results also converge with population-based cohort studies which show that childhood physical abuse is associated with having a parent with psychosis (Walsh et al., 2002; Fisher et al., in press).

Psychosocial stress and current psychopathology

The relationship between psychosocial stress and current psychopathology was found differ across the risk groups. Whilst PLEs were strongly associated with the frequency of daily hassles among ASz and FHx children, only moderate correlations were observed in the TD group; furthermore, PLEs were correlated with the number of negative life events among ASz children only. Negative life events and daily hassles have been associated with psychotic symptoms in previous studies of youth with a family history of illness (Miller et al., 2001) and adolescents with SPD (Tessner et al., 2011); however, correlations were examined across the total sample of high-risk youth and healthy controls. In contrast, daily hassles and life events experienced during the past month were not associated psychotic symptoms in a study of UHR youth (Thompson et al., 2007), which may reflect the shorter assessment period. Similar to previous studies in healthy children (Kliewer & Kung, 1998), significant correlations were observed between daily hassles and internalising and externalising symptoms in FHx and TD children. That this relationship was not also present in ASz children is consistent with a study reporting that daily hassles were not correlated with anxiety or depression among UHR youth (Thompson et al., 2007). Alternatively, this may reflect a 'ceiling effect' in measurement; it is possible that internalising and externalising symptoms among ASz children were sufficiently high so as to be unaffected by daily hassles.

The current study also investigated whether the relationship between physical punishment and psychopathology differed by risk group. Among FHx children, physical punishment at baseline was positively associated with internalising symptoms at follow-up, whilst current physical punishment experiences were associated with PLEs. In contrast, physical punishment was not correlated with any symptom measure in the ASz group; although current physical punishment was

associated with externalising symptoms in the TD group. Whilst this appears to be the first study to have examined the relationship between physical punishment and psychotic symptoms, a large epidemiological study observed a weak association between harsh parenting during early childhood (which included mild forms of physical punishment) and PLEs at 12 years (Fisher et al., 2013b). Furthermore, the correlations between physical punishment and internalising and externalising symptoms observed among FHx and TD children are highly consistent with existing literature on outcomes of physical punishment in childhood (Gershoff, 2002).

4.4.2 Potential mechanisms

Risk for schizophrenia and psychosocial stress exposure

In line with previous studies of youth at elevated risk for schizophrenia on account of their clinical presentation, ASz children were more frequently exposed to daily hassles than TD children. Consistent with the fact that eligibility for the ASz group at recruitment was partially determined on the basis of symptom presentation, ASz children continued to be characterised by higher scores on measures of internalising and externalising symptoms and PLEs at the 24-month follow-up (i.e., when psychosocial stress measures were completed). It is possible that these symptoms influence the way in which ASz children interact with their environment and increase the likelihood that they will encounter daily hassles in their lives. However, given the cross-sectional nature of these analyses, it is possible that ASz children were exposed to daily hassles prior to the onset of these symptoms. Indeed, psychosocial stress exposure may have triggered the emergence of PLEs in ASz children who may have been more susceptible to these experiences due to underlying biological vulnerability (as indexed by early neurodevelopmental abnormalities). Two recent population-based cohort studies of adolescents suggest that the relationship between stress exposure and schizophrenia vulnerability may be bidirectional (De Loore et al., 2007;

Kelleher et al., 2013c). Both studies found that traumatic experiences were associated with increased risk of developing PLEs, but that adolescents who reported PLEs at baseline were also at greater risk of being exposed to traumatic events during the follow-up period.

Relative to the TD group, FHx children reported a higher number of life events. As life events could not have feasibly contributed to FHx risk status (with the exception of a parent being seriously ill, which was reported by only 17% of the FHx group) it appears that having a family history of schizophrenia is associated with increased risk of experiencing negative life events. It is possible that having a close family member who is unwell (e.g., a parent or sibling) may create an environment where some of these events are more likely to occur. That this pattern was not observed in a study of older youth with a family history of schizophrenia (Miller et al., 2001), may be due to the fact that participants in the current study have more contact with their unwell family members due to their younger age.

Risk for schizophrenia and reactivity to psychosocial stress

ASz children reported that they were more distressed by the daily hassles they had experienced than TD children; one potential explanation for this finding is that these minor, frequently-occurring events are more salient to ASz children. Studies indicating that the dopamine system plays a key role in mediating the attribution of salience to environmental stimuli have led to the proposal that psychosis is a disorder of aberrant salience (Kapur, 2003). Hallucinations are proposed to result from the aberrant assignment of salience caused by hyperactive dopamine transmission whilst delusions are a compensatory mechanism that enables the individual to make sense of these experiences. Further extensions of this theory propose that salience dysregulation exists on a continuum in the population, often co-occurring with other features (e.g., mania and cognitive deficits), and that the need

for care arises if these symptoms reach a certain threshold (van Os, 2009). Thus, the heightened sensitivity to daily hassles among ASz children (who are already experiencing subclinical psychotic symptoms) may be due to underlying dopamine dysregulation which causes these events to be more salient.

In contrast to the heightened reactivity to daily experiences that characterised ASz children, FHx children reported feeling greater distress in relation to major negative life events relative to the TD group. However, significant group differences were observed only for current levels of distress, not for distress experienced at the time of the event. Major life events are, of course, distressing by nature, but experiencing a reduction in distress over time is likely to be an adaptive coping response. Indeed, all three groups reported that they were more distressed at the time of the event than they were currently. Thus, the significantly higher current distress ratings in FHx children relative to TD children may imply that FHx children used less adaptive coping mechanisms, causing their distress to persist at higher levels over time.

Role of ethnicity and socioeconomic status

The effect of ASz and FHx status on physical punishment was substantially reduced after adjusting for ethnicity and socioeconomic status; stratified analyses were therefore conducted to explore the role of these factors. Although limited by the small group sizes, these analyses suggest that in environments where physical punishment occurs less frequently (i.e., in families of white ethnicity or high socioeconomic status), ASz and FHx children are more likely to experience physical punishment than their TD peers. However, in environments where physical punishment is more regularly employed (i.e., non-white ethnic groups and low socioeconomic families), ASz and FHx children are no more likely to experience physical punishment, and in fact the TD group showed higher levels of exposure. These findings tentatively

suggest that factors such as socioeconomic status and ethnicity may moderate the relationship between risk for schizophrenia and physical punishment.

Relationship between psychosocial stress and psychopathology

Scores on the physical punishment scale were correlated with internalising symptoms and PLEs among FHx children only. In contrast, physical punishment was associated with externalising problems in the TD group (an outcome that has been reported consistently in the literature), but was not related to any measure of psychopathology in ASz children. Recent findings from a large, prospective study of children in the general population demonstrated that the relationship between childhood exposure to harsh parenting and PLEs in adolescence was mediated by anxiety, depressive symptoms, external locus of control, and low self-esteem (Fisher et al., 2013b). Thus, these psychological processes may lead to the expression of PLEs among FHx children, who may be particularly susceptible to the effects of physical punishment. Alternatively, the more robust associations between physical punishment and psychopathology observed among FHx children may reflect the fact that they were exposed to more severe forms of physical punishment (i.e., hitting with a belt, switch, or other object), which were not experienced by TD children. However, ASz children also experienced these more severe forms of punishment, so this cannot fully account for the findings.

4.4.3 Methodological considerations

Methodological issues that influence the overall study are discussed in detail in the final chapter of this thesis. Issues specifically relevant to the data presented in this chapter are described here. In common with previous research conducted in child and adolescent samples (Kliewer & Kung, 1998; Barrett & Heubeck, 2000; Tessner et al., 2011); a potential limitation is the reliance solely on self-report to assess daily hassles and negative life events. While subjective ratings of distress elicited by stress

exposure are inherent, exposure to negative life events and at least some daily hassles are amenable to obtaining confirmatory reports from caregivers. However, previous studies have shown poor concordance between parent and child ratings of life events and traumatic experiences (Stover et al., 2010; Tessner et al., 2011). Furthermore, the pattern of group differences in physical punishment and the association between these experiences and psychopathology was broadly similar across both child- and caregiver-reports. A further limitation of the current study is that it is not possible to disentangle the temporal relationship between psychosocial stress exposure and risk status. This is particularly problematic when trying to elucidate the relationship between psychosocial stress and ASz group membership. However, longitudinal population cohort studies of adolescents indicate that this relationship is likely to be bidirectional.

4.4.4 Conclusions

Children at elevated risk for schizophrenia are more frequently exposed to psychosocial stressors than their typically-developing peers and exhibit greater distress in relation to these experiences. These findings are consistent with previous studies showing that youth at elevated risk for schizophrenia on account of their clinical presentation are also more likely to be exposed to psychosocial stressors, and with studies showing that UHR youth and individuals with a family history of psychosis exhibit greater reactivity to stress. There is also evidence to suggest that experiences of psychosocial stress are more strongly associated with psychotic symptoms among children at elevated risk for schizophrenia compared to healthy controls. Overall, these findings are consistent with the predictions of the diathesis-stress model of schizophrenia. Chapters 5 and 6 will examine the extent to which susceptibility to psychosocial stress is associated with biological indices of HPA axis function among ASz and FHx children.

CHAPTER 5 Salivary cortisol in children at elevated risk for schizophrenia

5.1 Introduction

The HPA axis may play a key role in mediating the relationship between stress and psychosis (Walker & Diforio, 1997; Walker et al., 2008). Specifically, it is hypothesised that elevated cortisol levels (triggered by psychosocial stress exposure) contribute to the clinical features of psychosis by augmenting dopamine activity. Evidence to support this model has been obtained in studies examining cortisol levels in individuals with schizophrenia and healthy controls. Indeed, abnormal HPA axis function, as indexed by elevated diurnal cortisol levels and/or a blunted cortisol awakening response (CAR), has been observed among individuals who have recently experienced their first psychotic episode (Borges et al., 2013). These findings suggest that HPA axis abnormalities may be present during the early stages of illness. However, it is currently unclear as to whether HPA axis dysfunction emerges prior to the first psychotic episode or whether it is merely a consequence of the stress associated with illness onset.

Elevated salivary cortisol has been observed consistently among youth at UHR (Sugranyes et al., 2012; Walker et al., 2013) and adolescents with SPD (Weinstein et al., 1999; Walker et al., 2001; Mittal et al., 2007) relative to healthy youth. However, both higher (Collip et al., 2011; Yıldırım et al., 2011) and lower (Yang et al., 2012) cortisol levels during the day have been reported in individuals with a family history of psychosis, perhaps due to differences across studies in the cortisol sampling methodologies employed. Whilst these studies provide some evidence to suggest that elevated cortisol levels precede the onset of psychosis, UHR youth are, by definition, already sufficiently distressed as to seek treatment for their symptoms. Thus,

elevations in cortisol might feasibly be due to distress relating to emerging illness, as opposed to external psychosocial stressors. Furthermore, a substantial number of UHR youth are treated with antipsychotics and other psychotropic medications (Woods et al., 2013), which are known to affect cortisol levels. Similarly, studies of youth with SPD are limited by the fact that these individuals already present with symptoms of sufficient severity as to meet diagnostic criteria for a schizophrenia spectrum disorder. Examining cortisol levels in non help-seeking youth who are at an earlier stage of illness will help to establish whether abnormal HPA axis function precedes the onset of psychosis. Furthermore, it is also not yet known whether individuals at elevated risk for schizophrenia are also characterised by the blunted CAR that has been observed among individuals with first-episode psychosis.

Chapter aims

The aim of this chapter was to determine whether children presenting multiple antecedents of schizophrenia (ASz) and those with a family history of illness (FHx) are characterised by abnormal cortisol levels (elevated diurnal cortisol and/or a blunted CAR) relative to typically-developing (TD) children. A further aim was to determine whether cortisol levels are associated with experiences of psychosocial stress and current psychopathology among ASz and FHx children.

Hypotheses

2a. ASz children and FHx children will show elevated diurnal cortisol levels and a blunted CAR relative to TD children.

2b. Cortisol levels will be associated with exposure to psychosocial stressors and distress related to these exposures in ASz and FHx children.

2c. Among ASz and FHx children, cortisol levels will be correlated with current symptoms of psychopathology.

5.2 Methods

5.2.1 Participants and procedure

Full details of the recruitment procedure are provided in Chapter 3. In brief, ASz and TD children were identified using a novel community-screening procedure (Laurens et al., 2007; Laurens et al., 2011). FHx children were identified either via the caregiver screening questionnaire, or as relatives of individuals with schizophrenia or schizoaffective disorder. Children eligible for the ASz, FHx, and TD groups were invited to participate in a longitudinal study of child development. This chapter examines salivary cortisol data obtained at the 24-month follow-up assessment.

5.2.2 Salivary cortisol assessment

Sampling protocol

Participants were provided with a verbal explanation and written instructions for collecting saliva samples at home using a passive drool procedure (Salimetrics, Suffolk, UK) in which participants secreted saliva into 2ml cryovial storage tubes via a straw. Six saliva samples were collected throughout the day on two consecutive days: upon awakening (0 min), and at 15, 30, and 60 min after awakening, and at 12:00 pm and 20:00 pm. Sample collections were scheduled for weekends or school holidays in order to avoid interference with the child's school routine and maximise compliance. Participants were instructed to wake before 10:00 am and collect the first sample immediately upon awakening. Participants were also asked to avoid food consumption for 30 min prior to sample collection, and to refrain from strenuous exercise during the day. Participants recorded the actual times at which each sample was collected in a sampling diary as well as their activities prior to sampling and any food or drink consumed. Researchers ensured that sample collections were not scheduled on days when participants planned to engage in activities that might substantially affect cortisol levels (e.g., sports clubs, competitions, or sleepovers).

Processing and analysis

Samples were stored in the participant's home freezer until they were collected by the researcher and transported to the laboratory at the James Black Centre, Institute of Psychiatry. Samples were analysed using an established procedure described previously (Belvederi Murri et al., 2012). On arrival at the laboratory the samples were frozen at -20° C. Immediately before analysis, samples were thawed, and collection tubes were centrifuged for 15 min at 3000 rpm. Cortisol levels were determined using the Salimetrics High Sensitivity Salivary Cortisol ELISA KIT (Salimetrics, Suffolk, UK), following the recommended procedure. In brief, 25 µl of saliva and standards were assayed in duplicates by incubation on a microtitre plate coated with monoclonal antibodies against cortisol. Cortisol linked to horseradish peroxidase was added to compete with cortisol in the standards and unknowns for the antibody binding sites. After one hour of incubation, unbound components were washed away. Bound cortisol peroxidase was measured by reaction of the peroxidase enzyme on the substrate tetramethylbenzidine. The amount of cortisol peroxidase detected, as measured by the intensity of colour developed, was inversely proportional to the amount of cortisol present. Optical density was read at 450 nm with correction at 620 nm using a Beckman Coulter DTX 880 plate reader with Multimode Detection Software 2.0.0.12. Cortisol values were then calculated using SoftMax Pro 4.8 software, following a 4-parameter fit. All samples from the same participant were analysed in the same plate. The analytical sensitivity was set to 0.33 nmol/L, equivalent to 0.012 µg/dL. Inter-assay and intra-assay precisions ranged from 8% to 11% and 6% to 10%, respectively; these values are highly consistent those reported in other studies of youth at elevated risk for psychosis (Collip et al., 2011; Walker et al., 2013).

Computation of summary cortisol measures

Cortisol data obtained at individual time-points were summarised using the two area under the curve (AUC) computations described by Pruessner et al. (2003): (i) AUC with respect to the increase in cortisol levels following awakening (AUCi-CAR), and (ii) AUC with respect to ground of cortisol levels during the day (AUCg-DAY). Both computations are based on the trapezium formula, described in detail below.

AUCi-CAR values provide a measure of the CAR, that is, the increase in cortisol levels that typically occurs within the first 15-40 minutes after awakening (Pruessner et al., 1997). Cortisol values obtained from the awakening sample and the three post-awakening samples (15, 30, and 60 min) were used to derive an AUCi-CAR value for each participant. Firstly, the AUC with respect to ground (AUCg) was computed, reflecting the total amount of cortisol secreted during the hour after awakening. Secondly, the AUC with respect to the increase (AUCi) in cortisol levels from the first sample was computed by subtracting the area between the ground and the first cortisol value from the AUCg value. The equations for computing AUCg and AUCi values are provided below. Figure 7 shows the composition of these curves; time intervals (t) for the AUCi-CAR were: $t1$=15 min, $t2$=15 min, and $t3$=30 min.

Equation 3. Area under the curve with respect to ground (AUCg)

$$AUCg = \left(\left[\frac{(m2 + m1)t1}{2}\right]\right) + \left(\left[\frac{(m3 + m2)t2}{2}\right]\right) + \left(\left[\frac{(m3 + m4)t3}{2}\right]\right)$$

Equation 4. Area under the curve with respect to increase (AUCi)

$$AUCi = AUCg - ([t1 + t2 + t3]m1)$$

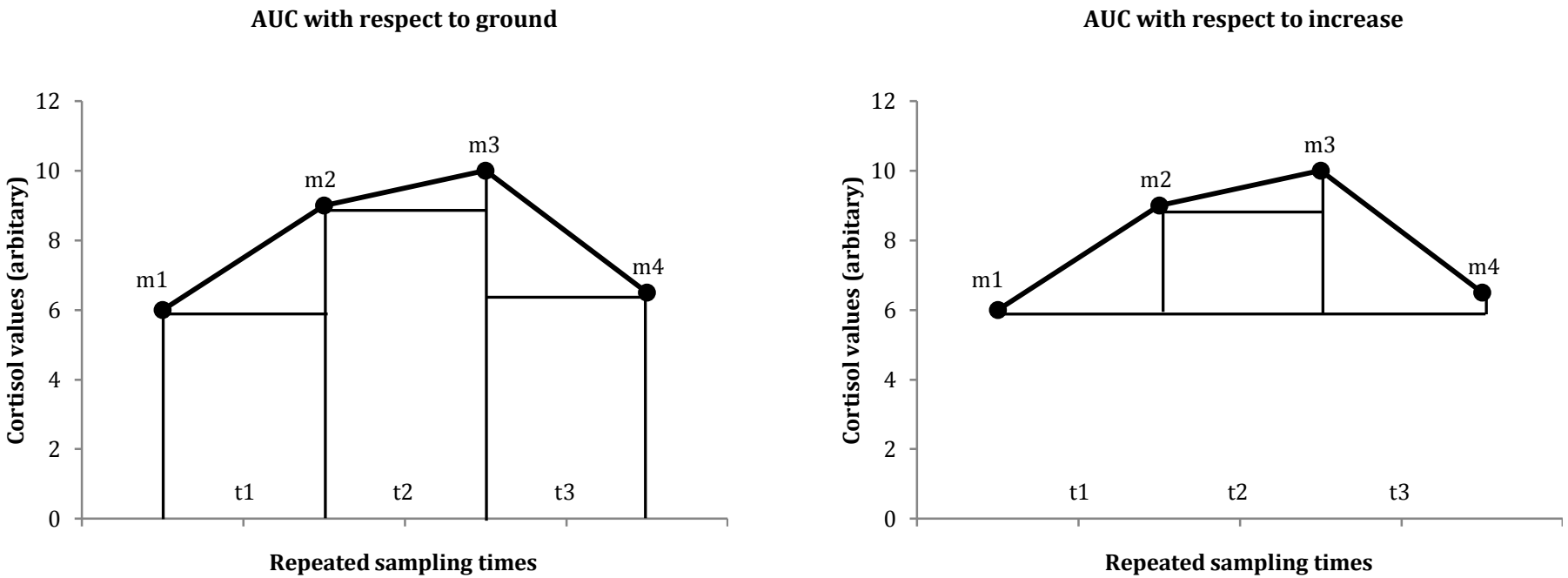

Figure 7. Composition of the area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) for the cortisol awakening response

Note. m: cortisol values at each sampling point; t: time interval between consecutive samples; t1: 15 min; t2: 15 min; t3: 30 min. Figures adapted from Pruessner et al. (2003).

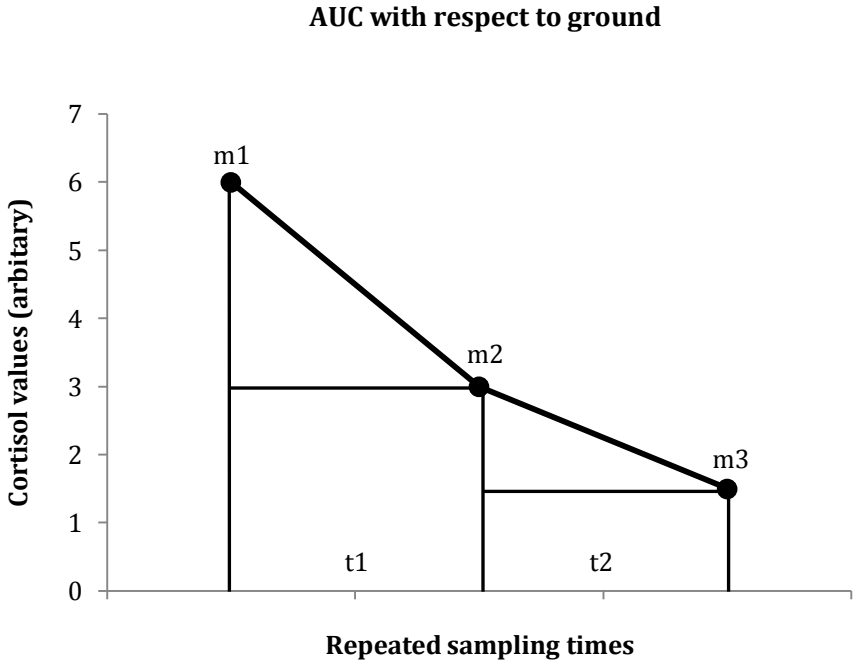

Figure 8. Composition of the area under the curve with respect to ground (AUCg) for cortisol during the day

Note. m: cortisol values at each sampling point; t: time intervals between consecutive samples; t1: time between the awakening and 12:00 pm samples; t2: time between the 12:00 pm and 20:00 pm samples. Figure adapted from Pruessner et al. (2003).

AUCg-DAY values, reflecting total cortisol levels during the day, were also computed using the formula provided above. To derive the AUC for cortisol during the day, it was necessary to compute time intervals (*t*) between consecutive sample measurements for each participant using the actual times at which the samples were collected. Thus, *t*1 corresponded to the time interval between the awakening sample and the actual time at which the 12:00 pm sample was collected (as determined by self-report), whilst *t*2 corresponded to the time interval between the actual times at which the 12:00 pm and 20:00 pm samples were collected. Figure 8 illustrates the composition of the AUCg-DAY curve.

Spearman's rank correlation analyses confirmed that cortisol values for each time-point were highly correlated across the two testing days (p<0.001 for each time-point). Thus, data obtained on the first sampling day only were used for AUC computations, unless Day 1 data were missing or participants demonstrated better compliance with the measurement protocol on Day 2 (e.g., if participants reported waking after 10:00 am or engaging in physical activities on Day 1 but not Day 2).

5.2.3 Psychosocial stress

Psychosocial stress measures were described in detail in Chapter 4. In brief, self-report measures were used to assess exposure and distress relating to a range of child-appropriate negative life events and school-related daily hassles (Heubeck & O'Sullivan, 1998). Physical punishment was assessed using the corporal punishment scale of the Alabama Parenting Questionnaire [APQ (Shelton et al., 1996)]. In order to assess physical punishment exposures occurring proximally to the salivary cortisol assessment, and to maintain consistency with the negative life event and daily hassles measures, this chapter examines child-reported physical punishment scale scores obtained at the 24-month follow-up. All psychosocial stress measures were completed within one month of collecting cortisol samples (mean lapse of time between questionnaire completion and cortisol assessments: ± 3.2 days).

5.2.4 Current psychopathology

Psychopathology measures were described in detail in Chapter 3. In brief, internalising and externalising symptoms were assessed via caregiver- and child-report using the Child Behaviour Checklist [CBCL (Achenbach & Rescorla, 2001)] and Youth Self-Report (YSR), respectively. Participants also re-completed the 9-item PLE measure (Laurens et al., 2012), with scores on each item summed to provide a total PLE score (range: 0-18).

5.2.5 Growth and pubertal status

Participant height and weight were collected by the researcher at the assessment session and used to compute Body Mass Index (BMI; kilograms/metres^2). Participants completed the Pubertal Developmental Scale (Carskadon & Acebo, 1993), a five-item self-report measure assessing three general domains of pubertal status: body growth, pubic hair, and skin changes; and two gender-specific domains: menstruation and breast growth (females), or voice changes and facial hair (males). Scores on each domain were averaged to create an overall score, with higher scores indicating more advanced pubertal development. Scores on three of these items were also summed (males: body hair growth, voice change, and facial hair; females: body hair, breast development, and menarche) and these scores were used to assign participants to one of the five Tanner stages (Tanner, 1962): pre-pubertal, early pubertal, mid-pubertal, late pubertal, and post-pubertal.

5.2.6 Tobacco and cannabis use

Tobacco and cannabis use were assessed using a self-report measure (McVie & Bradshaw, 2005) developed specifically for use in a large longitudinal study of adolescent substance use behaviours. To ascertain current tobacco use, participants were asked to chose one statement among five that best described their use ('I have never tried a cigarette', 'I have tried smoking cigarettes, but I don't smoke now', 'I smoke cigarettes, but less than once per week', 'I smoke cigarettes at least once a week', and 'I smoke cigarettes every day'). A further item asked participants to rate how often they had used cannabis ('never', 'once', '2-3 times', and '4 or more times'). Using established scoring criteria (McVie & Bradshaw, 2005), regular tobacco use was defined as having smoked 'at least once a week' or 'every day' and regular cannabis use as having used cannabis on 'at least four occasions'.

5.2.7 Statistical analyses

Group differences on demographic variables, psychosocial stress measures, and current psychopathology were examined using independent samples t-tests, Mann-Whitney U tests, chi-squared tests, and Fisher's exact tests. Independent samples t-tests, one-way ANOVAs, and correlation analyses were used to explore associations between cortisol AUC values and demographic variables.

As AUCi-CAR and AUCg-DAY values were approximately normally distributed, linear regression analyses were conducted to examine the effect of risk status on cortisol AUC measures. As previously, the effect of each risk group was tested independently (i.e., ASz and FHx groups were examined relative to the TD group but were not directly compared). All regression analyses were subsequently adjusted for demographic factors that differed significantly between the groups or that were associated with AUC values in preliminary analyses. Unstandardised regression coefficients were used to derive standardised mean differences (*d*) as indices of effect size (Lipsey & Wilson, 2001) in both adjusted and unadjusted analyses. As described in Chapter 3, where adjustment for demographic factors led to a substantial change in the effect size associated with risk status (change in $d > 0.30$), stratified analyses were performed to further explore the effect of these variables.

Within-group correlation analyses were conducted to examine the extent to which cortisol AUC values were associated with psychosocial stress and current psychopathology. Pearson's '*r*' correlation analyses were used for normally distributed variables (daily hassles scales, number of negative life events, and YSR scores) and Spearman's rho '*ρ*' correlation analyses for non-normally distributed variables (negative life event distress scales, APQ physical punishment scale scores, and CBCL and PLE scores). All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 20.

Table 20. Demographic characteristics of participants providing salivary cortisol data

	ASz (*n*=33)	FHx (*n*=22)	TD (*n*=40)	Statistics	
				ASz vs. TD	FHx vs. TD
Age (years); mean ± SE	12.8 ± 0.2	13.3 ± 0.3	13.1 ± 0.2	*t*=1.18 *p*=0.24	*t*=-0.50 *p*=0.62
Time lapse: screening to cortisol (years); mean ± SE	2.6 ± 0.1	2.7 ± 0.2	2.8 ± 0.1	*t*=1.05 *p*=0.30	*t*=0.22 *p*=0.83
Pubertal development scale score; mean ± SE [a]	2.4 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	*t*=-0.07 *p*=0.94	*t*=-0.19 *p*=0.85
BMI (kg/m^2); mean ± SE	20.4 ± 0.6	19.7 ± 0.7	19.8 ± 0.5	*t*=-0.76 *p*=0.45	*t*=0.09 *p*=0.93
Sex (male); *n* (%)	23 (70)	11 (50)	17 (43)	**χ^2=5.40 *p*=0.02**	χ^2=0.32 *p*=0.60
Tobacco use; *n* (%)	2 (6)	1 (5)	0 (0)	*FE* *p*=0.20	*FE* *p*=0.36
Ethnicity; *n* (%)				***FE*=7.69 *p*=0.05**	***FE*=17.46 *p*<0.001**
White British	8 (24)	3 (14)	20 (50)		
White other	8 (24)	2 (9)	11 (27)		
Black	4 (12)	8 (36)	3 (8)		
Other	13 (40)	9 (41)	6 (15)		
Socioeconomic status; *n* (%) [b]				***FE*=13.44 *p*=0.001**	***FE*=9.74 *p*=0.006**
Higher managerial, administrative, and professional	14 (43)	11 (50)	33 (82)		
Intermediate	12 (36)	5 (23)	6 (15)		
Routine and manual	7 (21)	6 (27)	1 (3)		
Time of awakening	8:36 ± 0:12	8:29 ± 0:13	8:34 ± 0:11	*t* =-0.11 *p*=0.79	*t*=0.27 *p*=0.91

Note. Four ASz+FHx cases are included in both groups. BMI: Body Mass Index; *FE*: Fisher's exact. [a] Mean scores in all groups equate to mid-pubertal stage. [b] Socioeconomic status based on caregiver occupation. Missing data: BMI (*n*=2).

5.3 Results

5.3.1 Sample characteristics

In total, 91 children completed the saliva collection protocol; 29 met ASz criteria only, 18 met FHx criteria only, 4 met both ASz and FHx criteria, and 40 met TD criteria. The four ASz+FHx cases were included in both the ASz and FHx groups, yielding data for 33 ASz and 22 FHx children in total. Demographic characteristics of the sample are presented by group in Table 20. There were no significant between-group differences (i.e., ASz vs. TD or FHx vs. TD) in age, pubertal status, lapse of time between antecedent screening and salivary cortisol assessment, BMI, tobacco use, or time of awakening (p>0.20). None of the participants reported regular cannabis use. The FHx and TD groups did not differ in sex; however, ASz children were significantly more likely to be male compared to TD children (p=0.02). Relative to the TD group, both the ASz and FHx group were found to differ significantly on ethnicity (p≤0.05) and socioeconomic status (p≤0.006).

5.3.2 Factors associated with salivary cortisol measures

Preliminary analyses were conducted in the total sample to identify sociodemographic factors associated with salivary cortisol. Analyses indicated that cortisol AUCi-CAR and AUCg-DAY values were not significantly associated with age, sex, ethnicity, pubertal status, tobacco use, or time of awakening (p≥0.10). However, there was a significant main effect of socioeconomic status on AUCg-DAY values (F[2, 82]=6.31, p=0.003); post-hoc tests indicated that children classed in the highest socioeconomic category (i.e., whose caregivers were employed in higher managerial, administrative, or professional occupations) had significantly lower AUCg-DAY values relative to children in the intermediate socioeconomic category (p=0.001).

5.3.3 Group differences in the cortisol awakening response

Figure 9 presents the mean cortisol values for the individual CAR time-points by group. Visual inspection of these data suggested that FHx children showed a smaller increase in cortisol during the first 15 minutes after awakening, and subsequently, a steeper decline in cortisol levels between 15 and 30 minutes compared to the TD group. In contrast, ASz children showed a similar pattern of post-awakening cortisol secretion to TD children. Mean AUCi-CAR values are presented in Table 21. Relative to the TD group, AUCi-CAR values were significantly lower in the FHx group (d=-0.73, p=0.01), reflecting a blunted CAR among FHx children. Post-hoc tests showed that magnitude of differences between FHx and TD children was twice as large among FHx children with a first-degree relative with schizophrenia (d=-1.09, p=0.005) compared to those with an affected second-degree relative (d=-0.50, p=0.14). Adjustment for demographic factors led to a substantial reduction in the effect of FHx status (d=-0.12, p=0.70). AUCi-CAR values did not differ between ASz and TD children (d=-0.19, p=0.42), with no change after adjustment for demographic factors.

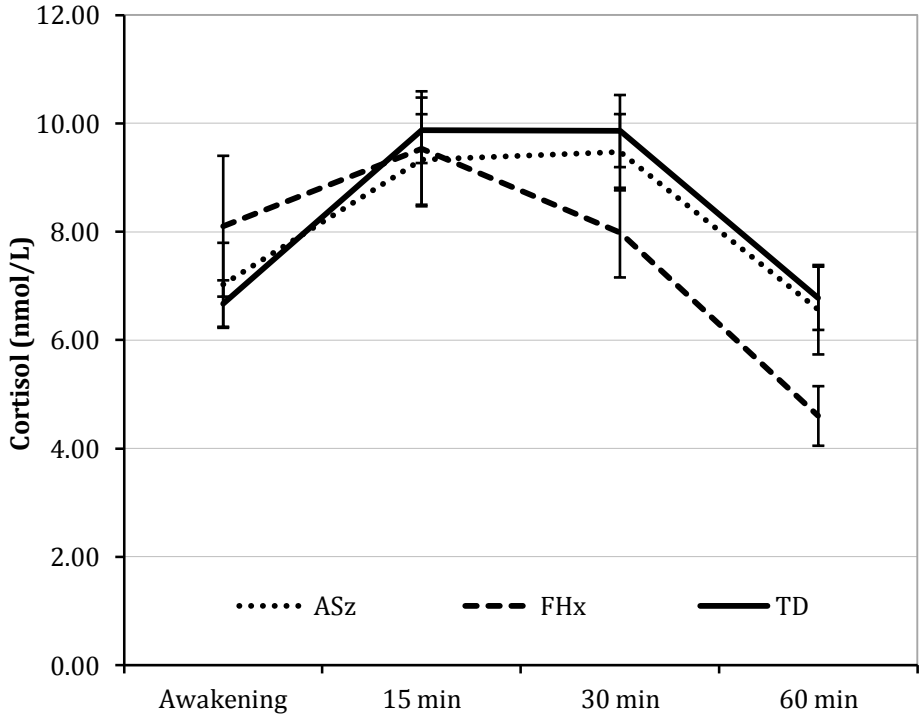

Figure 9. Mean (± SE) cortisol levels post-awakening by group

Table 21. Linear regression analyses examining the effect of risk status on the cortisol awakening response

	Descriptive statistics			Statistical analyses								
	ASz (*n*=33)	FHx (*n*=22)	TD (*n*=40)	Model [a]	ASz vs. TD				FHx vs. TD			
					d	*B*	(95% CI)	*p*	*d*	*B*	(95% CI)	*p*
AUCi-CAR (nmol min/L); mean ± SE	81.2 ± 39.0	-33.8 ± 52.5	121.6 ± 32.2	Unadjusted	-0.19	-40.4	(-140.3 – 59.5)	0.42	**-0.73**	**-155.3**	**(-272.2 – -38.4)**	**0.01**
				Adjusted	-0.08	-16.1	(-135.2 – 102.9)	0.79	-0.12	-26.2	(-161.6 – 109.2)	0.70

Note. Four ASz+FHx cases are included in both groups. *d*: standardised effect size; *B*: unstandardised regression coefficient. [a] Linear regression analyses without adjustment for potential confounders (unadjusted) and adjusted for sex, ethnicity, and socioeconomic status (adjusted).

Table 22. Stratified analyses examining risk status and the cortisol awakening response

	AUCi-CAR values (nmol min/L); mean ± SE												
	ASz (*n*=33)	FHx (*n*=22)	TD (*n*=40)	Statistics *					ASz (*n*=33)	FHx (*n*=22)	TD (*n*=40)	Statistics *			
				ASz vs. TD		FHx vs. TD						ASz vs. TD		FHx vs. TD	
				d	*p*	*d*	*p*					*d*	*p*	*d*	*p*
Male	56.7 ± 28.4	-38.5 ± 61.2	56.4 ± 26.5	0.00	1.00	-0.62	0.18	White	53.4 ± 68.9	136.5 ± 47.5	139.4 ± 38.7	-0.37	0.25	-0.01	0.98
Female	143.7 ± 120.9	-28.5 ± 91.1	169.7 ± 50.6	-0.09	0.82	**-0.77**	**0.05**	Other	105.6 ± 42.5	-87.0 ± 62.1	60.3 ± 49.3	0.27	0.52	-0.67	0.12

Note. *d*: standardised effect size. * Independent samples t-tests.

Sex and ethnicity were significant predictors of AUCi-CAR values in multiple regression analyses and their inclusion in the model substantially reduced the effect of FHx status. Stratified analyses were therefore conducted to further examine the influence of these variables on the relationship between risk status and the CAR (Table 22). The pattern of lower AUCi-CAR values among FHx children relative to TD children was present in both males and females with moderate-to-large effect sizes (d=-0.62 and -0.77, respectively), although group differences only achieved statistical significance in females. In contrast, in both males and females, AUCi-CAR values did not differ between ASz and TD children (p>0.82). As previously, ethnicity groups were combined to derive two summary groups: 'white' (including 'white British' and 'white other') and 'other' (including 'black' and 'other'). Within the 'other' ethnicity group, FHx children continued to show lower AUCi-CAR values compared to the TD group, equating to a moderate effect size (d=-0.67, p=0.12). However, among children of 'white' ethnicity, FHx and TD children did not differ on AUCi-CAR values (d=-0.01, p=0.98). There were no differences between ASz and TD children on AUCi-CAR values when 'white' or 'other' groups were examined separately (p>0.25).

5.3.4 Group differences in diurnal cortisol

Mean cortisol values at awakening, 12:00 pm, and 20:00 pm are presented by group in Figure 10. When these data were inspected visually, all three groups appeared to exhibit an identical pattern of secretion throughout the day, with cortisol levels showing a sharp decrease between awakening and 12:00 pm, followed by a more gradual decline between midday and 20:00 pm. Mean AUCg-DAY values are presented by group in Table 23. Linear regression analyses indicated that there were no group differences in AUCg-DAY values when either ASz (d=0.21, p=0.38) or FHx groups (d=0.08, p=0.78) were compared to the TD group; results were unchanged after adjusting for demographic variables.

Table 23. Linear regression analyses examining the effect of risk status on diurnal cortisol

	Descriptive statistics			Statistical analyses								
					ASz vs. TD				FHx vs. TD			
	ASz (*n*=33)	FHx (*n*=22)	TD (*n*=40)	Model [a]	*d*	*B*	(95% CI)	*p*	*d*	*B*	(95% CI)	*p*
AUCg-DAY (nmol h/L); mean ± SE	36.59 ± 3.14	34.47 ± 2.99	33.54 ± 1.79	Unadjusted	0.21	3.05	(-3.89 – 9.99)	0.38	0.08	0.92	(-5.71 – 7.56)	0.78
				Adjusted	0.13	1.90	(-5.87 – 9.67)	0.63	-0.29	-3.24	(-10.87– 4.38)	0.40

Note. Four ASz+FHx cases are included in both groups. *d*: standardised effect size; *B*: unstandardised regression coefficient. [a] Linear regression analyses without adjustment for potential confounders (unadjusted) and adjusted for sex, ethnicity, and socioeconomic status (adjusted).

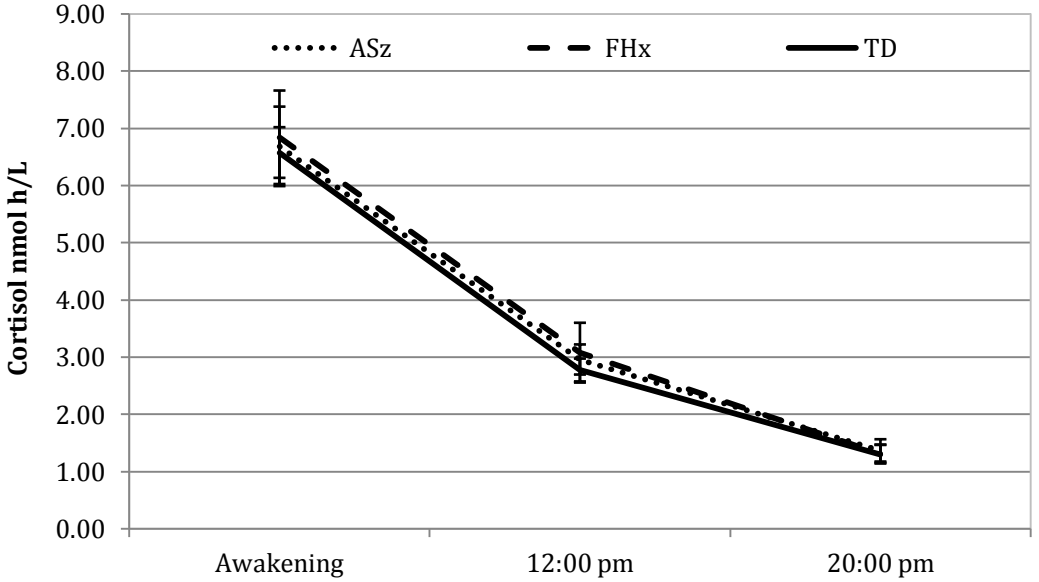

Figure 10. Mean (± SE) diurnal cortisol levels by group

5.3.5 Cortisol and psychosocial stress

Consistent with the data presented in Chapter 4, relative to the TD group, ASz and FHx children experienced a higher number of negative life events (p=0.007 and p=0.08, respectively) and obtained higher scores on the physical punishment scale p<0.005). ASz children also demonstrated higher scores on the total daily hassles frequency and distress scales (p≤0.001). Within-group correlation analyses were conducted to examine relationships between cortisol AUC values and psychosocial stress measures (Table 24). Across all three groups, AUCi-CAR values were not significantly associated with the total number of negative life events experienced (p≥0.30). However, among FHx children, AUCi-CAR values were *positively* correlated with the level of distress experienced in relation to negative life events at the time of the event (ρ=0.51, p=0.02) and with the level of distress experienced currently (ρ=0.52, p=0.02). In contrast, among TD children, AUCi-CAR values were *negatively* correlated with distress experienced at the time of the negative life event (ρ=-0.31, p=0.05). These variables were not significantly correlated among ASz children. AUCi-CAR values were not associated with daily hassles or physical punishment, and none of the psychosocial stress measures were correlated with AUCg-DAY values (p≥0.10).

Table 24. Correlations between cortisol and psychosocial stress

	AUCi-CAR			AUCg-DAY		
	ASz (n=33)	FHx (n=22)	TD (n=40)	ASz (n=33)	FHx (n=22)	TD (n=40)
Total No. negative life events	0.03	0.21	-0.03	0.09	0.04	0.27
Life event previous distress	-0.16	0.51*	-0.31*	0.30	0.06	0.11
Life event current distress	-0.05	0.52*	-0.27	-0.12	-0.05	0.20
Daily hassles frequency scale	0.13	0.18	-0.12	0.10	0.00	0.18
Daily hassles distress scale	0.16	0.17	0.00	-0.26	0.16	0.20
Physical punishment	-0.03	-0.35	-0.11	-0.11	0.31	0.05

Note. * p<0.05.

5.3.6 Cortisol and current psychopathology

As previously (Chapter 4), ASz children were characterised by significantly higher scores on the CBCL internalising and externalising scales, the YSR externalising scale, and the PLE measure relative to the TD group (p<0.01). FHx children were not significantly different to the TD group on internalising or externalising symptoms (either CBCL or YSR), but obtained higher scores on the PLE measure (p=0.04). Within-group correlation analyses were conducted to examine associations between cortisol AUC values and current psychopathology (Table 25). Across all three groups, AUCi-CAR and AUCg-DAY values were not significantly correlated with internalising or externalising symptoms (either child- or caregiver-reported). AUCi-CAR values were moderately correlated with PLE scores in the FHx group, although this did not achieve statistical significance (ρ=0.37, p=0.10). Cortisol AUC values were not associated with PLEs in ASz children or in the TD group.

Table 25. Correlations between cortisol and current psychopathology

	AUCi-CAR			AUCg-DAY		
	ASz (n=33)	FHx (n=22)	TD (n=40)	ASz (n=33)	FHx (n=22)	TD (n=40)
YSR Internalising scale	0.00	0.19	0.03	0.12	-0.11	0.16
YSR Externalising scale	-0.04	0.14	-0.17	-0.07	-0.22	0.23
CBCL Internalising scale	-0.15	-0.01	0.15	0.09	0.21	0.07
CBCL Externalising scale	-0.15	0.08	-0.08	0.06	0.21	0.26
Psychotic-like experiences	0.12	-0.37	-0.03	0.17	-0.08	-0.26

Note. p>0.05 for all correlations. YSR: Youth Self-Report; CBCL: Child Behaviour Checklist.

5.4 Discussion

In the first study to examine the CAR in individuals at elevated risk for schizophrenia, children with a family history of illness, but not children presenting antecedents of schizophrenia, were characterised by a blunted CAR relative to their typically-developing peers; an effect that appeared to be influenced by ethnicity. However, neither FHx nor ASz children were characterised by elevated diurnal cortisol levels relative to the TD group. The blunted CAR observed among FHx children was not explained by experiences of psychosocial stress; indeed, the CAR was positively correlated with distress relating to negative life events among FHx children but negatively correlated in the TD group. Neither the CAR nor cortisol levels during the day were associated with daily hassles or physical punishment. Furthermore, cortisol levels were not related to current psychopathology in any group.

5.4.1 Comparison with previous research

Cortisol awakening response

The finding that FHx children were characterised by a blunted CAR relative to TD children converges with two recent studies which observed a blunted CAR in patients with first-episode psychosis compared to healthy controls (Mondelli et al., 2010a; Pruessner et al., 2013b). Adjustment for demographic factors indicated that sex and ethnicity may influence the relationship between risk status and the CAR; stratified analyses subsequently confirmed that the pattern of group differences was consistent across males and females but not across ethnic groups. In healthy adult and adolescent populations, lower cortisol levels upon awakening have been reported among individuals of black and Hispanic ethnicity relative to white participants (DeSantis et al., 2007; Hajat et al., 2010). Whilst these findings raise the possibility that the blunted CAR observed among FHx children might be due to the fact that these children were more likely to be of non-white ethnicity, this was not the case, in

fact, FHx status was still associated with a blunted CAR (with a moderate-to-large effect size) when the analysis was restricted to non-white participants. In contrast, there was no difference in the CAR when white FHx and white TD children were compared, which may reflect the small group sizes. Among the five FHx children who were of white ethnicity, only one had a first-degree relative with the disorder, whilst 8 of the 17 non-white FHx children had an affected first-degree relative. Given that the effect of FHx status was twice as large among those with a first-degree relative with schizophrenia compared to those with a second-degree relative, this may have also contributed to the lack of group differences among children of white ethnicity.

Diurnal cortisol

In contrast, neither FHx nor ASz children were characterised by elevated diurnal cortisol levels relative to TD children, which contrasts with the elevated salivary cortisol levels reported among adult relatives of patients with psychosis (Collip et al., 2011; Yıldırım et al., 2011), youth at UHR (Sugranyes et al., 2012; Walker et al., 2013), adolescents with SPD (Walker et al., 2001; Mittal et al., 2007), and youth reporting PLEs (Mittal et al., 2013). This may be due to the participants being slightly younger (mean age: 13.1 years) than high-risk youth in previous studies (mean age range: 14.1-39.3 years). Longitudinal studies indicate that cortisol levels during the day increase during late childhood and through adolescence (Walker et al., 2001; Shirtcliff et al., 2012), thus, the elevation in diurnal cortisol levels observed in high-risk youth relative to their healthy peers may not emerge until later in adolescence.

Cortisol and psychosocial stress

Cortisol levels were not correlated with the number of life events experienced or with the frequency of daily hassles in any group. A previous study of UHR youth also observed no relationship between the number of life events and cortisol, although cortisol levels were positively associated with the number of daily hassles

(Thompson et al., 2007). One possible explanation for the lack of association observed between cortisol levels and daily hassles in the current study is that the latter were not always assessed on the day of cortisol collection. For example, a large study of adults which obtained concurrent measures of salivary cortisol and daily hassles, reported that cortisol levels were significantly higher on days when participants experienced daily stressors compared to stress-free days (Stawski et al., 2013). In contrast, among FHx and TD children, the CAR was correlated with distress relating to negative life events (although, as discussed below, the pattern of association was different across the two groups). The negative association between the CAR and distress relating to negative life events that was observed among TD children is consistent with previous studies which have reported that higher levels of subjective distress relating to chronic stressors are associated with lower morning cortisol levels (Miller et al., 2007).

Cortisol and current psychopathology

Cortisol levels were not significantly correlated with PLEs in ASz, FHx, or TD children. Studies of UHR youth have also observed that cortisol levels are not associated with psychotic or prodromal symptoms (Thompson et al., 2007; Corcoran et al., 2012; Sugranyes et al., 2012). In contrast, the largest study of UHR youth reported that salivary cortisol levels were significantly correlated with positive and negative psychotic symptoms (Walker et al., 2013). Furthermore, two studies examining adolescents with SPD, other personality disorders, and no personality disorders observed that salivary cortisol levels were associated with negative symptoms (Weinstein et al., 1999) and SPD symptoms (Walker et al., 2001). However, this may reflect the fact that correlations were performed across the total sample of healthy and at-risk youth (i.e., the fact that these groups differ on both cortisol levels and psychopathology may have contributed to the association).

The finding that cortisol levels were unrelated to internalising and externalising symptoms contrasts with previous studies of children and adolescents which have typically shown that elevated cortisol during the day is positively associated with depression (Lopez-Duran et al., 2009) and negatively correlated with externalising problems (Susman, 2006). Similarly, cortisol levels were found to be positively correlated with depression and anxiety symptoms in two previous studies of UHR youth (Thompson et al., 2007; Corcoran et al., 2012). However, not all studies of children and adolescents have observed this pattern, and it has been suggested that this may be due to studies not accounting for sex and symptom comorbidity (Marsman et al., 2008). Thus, the lack of significant associations in the current study may reflect the fact that correlations were examined across males and females and were not adjusted for the presence of other symptoms.

5.4.2 Potential mechanisms

Genetic influence on the CAR

One possible explanation for the fact that FHx children, but not ASz children, were characterised by a blunted CAR relative to the TD group is that this reflects a genetically-mediated effect. For example, a study of individuals at increased risk of developing PTSD on account having parents with the disorder observed lower cortisol levels among offspring (a consistent finding among individuals with PTSD) even though they had not been exposed to trauma themselves (Yehuda et al., 2000). The fact that the effect of FHx status was twice as large among those with a first-degree relative with schizophrenia than among those with a second-degree relative is consistent with this hypothesis (although, children with a first-degree relative are also likely to be living with their affected relative, which may in itself be more stressful). That FHx children were not also characterised by elevated diurnal cortisol levels may be due to the fact that the CAR is under more genetic control. Indeed, twin

studies in both adult and adolescent populations indicate that cortisol levels upon awakening and during the morning (Bartels et al., 2003) and the CAR (Wust et al., 2000) are influenced by genetic factors, whilst cortisol levels later in the day are not.

Of note, the fact that distress relating to negative life events was positively and not negatively correlated with the CAR among FHx children, suggests that the greater levels of distress experienced by FHx children relative to TD children cannot account for the blunted CAR observed among FHx children. Again, this is consistent with a previous study which observed that the CAR was positively correlated with the presence of childhood trauma among patients with first-episode psychosis (Mondelli et al., 2010a). Taken together, these lines of evidence indicate that the blunted CAR that has been described both in patients with psychosis (Mondelli et al., 2010a), and, in the current study, among children at elevated risk for the disorder, is not driven by the excess of psychosocial stressors but rather may reflect the expression of a genetic predisposition.

Effect of psychosocial stress on cortisol levels

The current study identified a dissociation between psychosocial stress and cortisol among FHx and TD children; distress relating to negative life events was negatively correlated with the CAR in the TD group whilst a positive correlation was observed among FHx children. As described above, the same pattern was observed between the CAR and childhood trauma in a sample of individuals with first-episode psychosis (Mondelli et al., 2010a); furthermore, this study also reported a negative correlation between diurnal cortisol and the number of recent stressful events in the patient group but a positive correlation among healthy controls. The different pattern observed among FHx and TD children may be influenced by ethnicity; for example, cortisol responses to psychosocial stress and perceived discrimination have been found to differ across ethnic groups (Chong et al., 2008; Suglia et al., 2010; Fuller-

Rowell et al., 2012). However, ASz children were also significantly different to TD children on ethnicity, but did not show the same pattern of association between the CAR and distress relating to negative life events as FHx children. Furthermore, although it has been observed that the lapse of time between stressor exposure and cortisol measurement influences the extent to which morning cortisol levels are positively or negatively associated with stress exposure (Miller et al., 2007), the negative life events experienced by TD children did not occur more recently than those experienced by FHx children. Thus, one possible explanation for the observed dissociation is that psychosis (and psychosis vulnerability) influences the effect of psychosocial stress on HPA axis function. This may relate to how these stressful events are appraised; for example, the degree to which stressors are viewed as potentially controllable or uncontrollable has been associated with higher and lower morning cortisol levels, respectively (Miller et al., 2007). Alternatively, genetic mechanisms may have contributed to the divergent pattern of findings observed among FHx and TD children.

5.4.3 Methodological considerations

As previously, this section describes the methodological issues that are relevant to the data presented in this chapter only. A more comprehensive discussion of the issues that influence the overall study is provided in the final chapter of this thesis. One potential limitation relates to the fact that saliva samples were collected at weekends or during school holidays to improve the likelihood of protocol compliance. Studies in adults have shown that the CAR is more pronounced on weekdays compared to weekends (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004); however, as all participants collected samples at weekends or during school holidays, this would not affect group differences (although, it is possible that the unstructured nature of the homes of FHx children at weekends was more pronounced than in TD

children). As acknowledged above, a further limitation relates to the fact that psychosocial stressors (particularly daily hassles) were not always assessed on the day of cortisol collection. Again, as the groups did not differ on the lapse of time between psychosocial stress assessment and saliva sample collection, this would have affected all groups equally. Finally, the study was not able to examine the relationship between HPA axis function and more severe forms of psychosocial stress such as childhood maltreatment, which has been implicated in the development of psychosis (Schafer & Fisher, 2011) and associated with a blunted cortisol response to stress (Ouellet-Morin et al., 2011).

5.4.4 Conclusions

The current study provides evidence that HPA axis dysfunction, as indexed by a blunted CAR, characterises children at elevated risk for psychosis who are not currently exhibiting help-seeking behaviour. That this was only observed among FHx and not ASz children, and was more pronounced among FHx children with a first-degree relative with schizophrenia, raises the possibility that the blunted CAR may be a genetically-mediated effect; although environmental factors may also have contributed to this finding. In contrast to the findings in older samples of UHR youth and adolescents with SPD, neither FHx nor ASz children displayed elevated diurnal cortisol levels. Thus, the blunted CAR may be an early (possibly genetically-mediated) marker of HPA axis dysfunction, whilst elevated diurnal cortisol levels may emerge later, closer to disease onset. Longitudinal follow-up of this cohort is necessary to confirm this hypothesis. The results described in this chapter are consistent with previous studies of patients with first-episode psychosis, and thus further support the notion that at least some HPA axis changes precede the onset of illness, rather than being a subsequent epiphenomenon.

CHAPTER 6 Pituitary volume in children at elevated risk for schizophrenia

6.1 Introduction

Pituitary gland volume has also been used as an index of HPA axis function among individuals with psychosis. Cross-sectional studies have reported enlarged pituitary volumes in individuals with first-episode psychosis relative to healthy controls (Pariante et al., 2004; Pariante et al., 2005; Büschlen et al., 2011; Takahashi et al., 2011), and smaller volumes among patients with established schizophrenia (Pariante et al., 2004; Upadhyaya et al., 2007). However, not all studies have observed this pattern of findings (Nicolo et al., 2010; Gruner et al., 2012; Klomp et al., 2012), and inconsistencies may relate to differences in illness stage and antipsychotic use.

Studies examining individuals at elevated risk for schizophrenia have also yielded conflicting findings, which may again relate to differences in medication use and proximity to illness onset. Relative to healthy controls, enlarged pituitary volume has been observed among adults with a family history of schizophrenia (Mondelli et al., 2008) and patients with SPD (Takahashi et al., 2009). However, other studies have shown no differences between adult relatives and controls (Habets et al., 2012), and smaller pituitary volumes among males with SPD (Romo-Nava et al., 2013). Studies of youth at UHR indicate that pituitary volumes across the total sample of UHR youth do not differ to healthy controls (Garner et al., 2005; Büschlen et al., 2011), but that those who later transition to psychosis show enlarged pituitary volumes. Whilst these studies provide some evidence to suggest that high-risk individuals are characterised by abnormal pituitary volume, the extent to which this reflects stress-induced HPA axis dysfunction is unclear. Previous studies of young adults with a family history of psychosis (Habets et al., 2012) and youth at UHR (Thompson et al.,

2007) have observed no relationship between pituitary volume and cortisol during the day. Furthermore, the only study of high-risk individuals to examine the relationship between pituitary volume and psychosocial stress reported only a weak association between pituitary volume and emotional stress reactivity (Habets et al., 2012). However, pituitary volume has been associated with exposure to childhood maltreatment in youth with PTSD and borderline personality disorder (Thomas & De Bellis, 2004; Garner et al., 2007). One consistent finding across all high-risk groups is that pituitary volumes do not appear to be associated with psychotic symptoms.

Chapter aims

This chapter examines pituitary gland volume in medication-naive children at elevated risk for schizophrenia. The aim was to determine whether children presenting multiple antecedents of schizophrenia (ASz) and those with a family history of illness (FHx) are characterised by abnormal pituitary volumes relative to typically-developing (TD) children. An additional aim was to determine the extent to which pituitary volume is associated with cortisol levels and experiences of psychosocial stress among at-risk children.

Hypotheses

3a. Both ASz and FHx children will show abnormal pituitary gland volume relative to TD children.

3b. Among ASz and FHx children, pituitary volume will be correlated with salivary cortisol levels.

3c. Pituitary volume will be associated with exposure to psychosocial stressors and distress related to these exposures in ASz and FHx children.

3d. No relationship between pituitary volume and current psychopathology will be observed among ASz and FHx children.

6.2 Methods

6.2.1 Participants and procedure

As described in detail in Chapter 3, a novel community-screening procedure was used to identify ASz and TD children (Laurens et al., 2007; Laurens et al., 2011). FHx children were identified either via the caregiver screening questionnaire, which included items assessing family mental health difficulties, or as child relatives of individuals with schizophrenia or schizoaffective disorder identified via medical records. ASz, FHx, and TD children were invited to participate in a longitudinal study of child development. This chapter examines pituitary volume obtained from structural MRI scans collected at the 24-month assessment.

6.2.2 Pituitary gland measurement

Image acquisition

Participants completed a one hour MRI scanning session which included a structural scan and four functional MRI tasks. Scans were conducted on a 3T General Electric Medical Systems MRI scanner (General Electric, Milwaukee, Wisconsin, USA) based at the Centre for Neuroimaging Sciences, Institute of Psychiatry. An 8-channel coil was used for radio detection frequency. Structural images were acquired using a five minute, three-dimensional Spoiled Gradient Recalled Echo (SPGR) sequence yielding 196 slices of 1.1 mm thickness. Imaging parameters were as follows: time-to-repetition = 6.0 msec, time-to-echo = 2.8 msec, flip angle = 20°, field-of-view = 28 x 21 cm, acquisition matrix = 256 x 256.

Measure software and image processing

Pituitary gland volume was obtained using Measure (version 0.8, John Hopkins University, Baltimore, MD, USA), a structural MRI processing and analysis software programme. Anonymised scans were imported into Measure and the pituitary gland

was then manually traced on each scan using the stereological Cavalieri method employing a point counting procedure. In brief, the Cavalieri method uses parallel plane sections, beginning at a randomly chosen start point, to create equally-spaced sections throughout the brain; the surface area on each slice is then estimated and multiplied by the section thickness to provide a volume for the structure (Sonmez et al., 2010). Appling the Cavalieri method in conjunction with the point counting procedure is considered to be an unbiased method and more reliable and efficient than traditional region-of-interest drawing techniques (Howard et al., 2000).

Structural MRI data were processed according to an established procedure (Measure Standardised Operating Procedures, Institute of Psychiatry, London, UK). Images were first aligned to the anterior commissure - posterior commissure (AC-PC) line and standard contrast settings applied. For each scan slice, a grid of pixels was then superimposed on the image and pixels that fell within the pituitary gland (defined as more than 60% of the pixel falling within the boundary of the structure) were selected. Figure 11 shows the pituitary gland, as visualised in Measure, in the coronal, axial, and sagittal view. Measure guidelines specify that the minimum number of pixels needed to reliably estimate the volume of a given structure is 200, thus, the smallest grid setting (1x1x1) was used in order to ensure sufficient pixels within the pituitary gland. Traces were performed in the coronal view, using information from the sagittal and axial view for clarification. Pixels were selected on each slice on which the pituitary gland could be visualised. Total volumes (mm^3) were computed using a macro function operating within a Microsoft Excel spreadsheet.

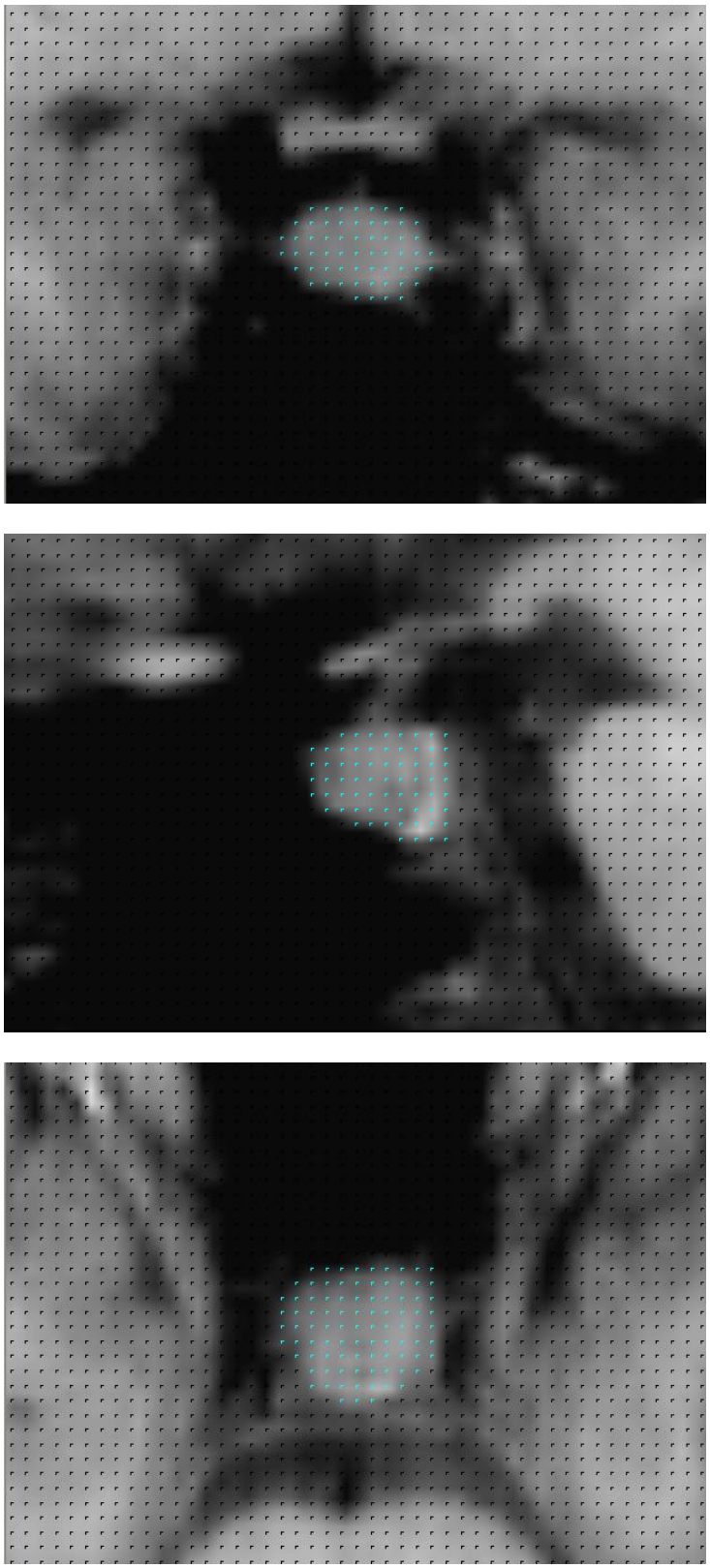

Figure 11. The pituitary gland as visualised in Measure

Note. Coronal (top), sagittal (middle), and axial (bottom) view of a structural MRI image in Measure with superimposed pixel grid. Pixels falling within the boundaries of the pituitary gland are highlighted in blue.

Anatomical boundaries

Anatomical boundaries of the pituitary gland were determined using a protocol implemented in previous studies (Pariante et al., 2004; Pariante et al., 2005; Mondelli et al., 2008). The measurements excluded the pituitary stalk but included the posterior bright spot (where visible) which is thought to correspond to the posterior pituitary. The superior and inferior boundaries of the pituitary were defined by the diaphragma sellae and the sphenoid sinus, respectively. The cavernous sinuses were used to indicate the lateral boundaries. Training was provided by an experienced researcher (FD, see acknowledgements) with expertise in tracing the pituitary gland in youth at UHR for psychosis. The inter-rater reliability (based on 10 scans) was 0.97, indicating a high level of reliability with other raters.

Total intracranial volume

Total intracranial volume (ICV) was obtained using Statistical Parametric Mapping 5 software (SPM5; www.fil.ion.ucl.ac.uk/spm/software/spm5). Images were first segmented in SPM5 using a study-specific template created within the Template-O-Matic (TOM) toolbox (https://irc.cchmc.org/software/tom.php). Specifically, brain images used to create the template were derived from reference data obtained from 404 healthy children (Wilke et al., 2008), who were selected according to the age and sex characteristics of the total sample of participants in the current study. Total grey matter, white matter, and cerebrospinal fluid volumes were obtained from segmented images and summed to derive a total ICV for each participant.

6.2.3 Salivary cortisol

Salivary cortisol assessments were completed within six months of scanning (mean lapse of time assessments: ± 0.9 months). Full details of the cortisol sampling protocol are provided in Chapter 5. In brief, participants collected six saliva samples throughout the day on two consecutive days: upon awakening, at 15, 30, and 60 min

after awakening, and at 12:00 pm and 20:00 pm. Cortisol data obtained at individual time-points were summarised using two area under the curve (AUC) computations (Pruessner et al., 2003): (i) AUC with respect to the increase in cortisol levels following awakening (AUCi-CAR), and (ii) AUC with respect to ground of cortisol levels during the day (AUCg-DAY).

6.2.4 Psychosocial stress

As described in Chapter 4, participants completed measures assessing exposure and distress relating to a range of child-appropriate negative life events and school-related daily hassles (Heubeck & O'Sullivan, 1998). Physical punishment was assessed using the corporal punishment scale of the Alabama Parenting Questionnaire [APQ (Shelton et al., 1996)], completed by children and their caregivers at both the baseline and 24-month assessment phases. Whilst salivary cortisol levels show rapid fluctuations in response to environmental stimuli, pituitary volume is thought to provide a more stable measure of HPA axis activity; thus, in order to provide a more comprehensive index of exposure to physical punishment, both baseline and 24-month assessment data were examined in this chapter. As previously described, a dichotomous variable was created from the three physical punishment items (spanking, slapping, and hitting with an object) in order to incorporate information from both informants (child and caregiver) and both assessment phases (baseline and 24-month).

6.2.5 Current psychopathology

As described in Chapter 3, externalising and internalising symptoms were assessed using the Child Behaviour Checklist [CBCL (Achenbach & Rescorla, 2001)] and the Youth Self-Report (YSR), completed by the caregiver and the child, respectively. Children also re-completed the nine-item PLE measure (Laurens et al., 2012), with scores on each item summed to provide a total PLE score (range: 0-18).

6.2.6 Growth and pubertal status

Body Mass Index (BMI; kilograms/metres^2) was computed from height and weight data obtained by a researcher at the MRI session. As described in Chapter 5, pubertal development was assessed using the self-report Pubertal Developmental Scale (Carskadon & Acebo, 1993). An average score was created from the five items, with higher scores indicating more advanced pubertal development. Scores on three of the items were summed and used to assign participants to a Tanner stage (Tanner, 1962) and a dichotomous variable was subsequently created (pubertal: Tanner stage 1-4 vs. post-pubertal: Tanner stage 5), based on previous studies (Ekelund et al., 2006).

6.2.7 Statistical analyses

Group differences on demographic variables, cortisol levels, psychosocial stress measures, and current psychopathology were examined using independent samples t-tests, Mann-Whitney U tests, chi-squared tests, and Fisher's exact tests. Independent samples t-tests, one-way ANOVAs, and correlation analyses were used to explore associations between pituitary volume and demographic variables.

As pituitary gland volumes were approximately normally distributed, the effect of risk status on pituitary gland volume was examined using linear regression analyses. As previously, the effect of each high-risk group was tested independently (i.e., ASz and FHx groups were examined relative to the TD group but were not directly compared to each other). Analyses were subsequently adjusted for factors that were associated in preliminary analyses with group status or pituitary volume. Unstandardised regression coefficients from both unadjusted and adjusted analyses were used to derive standardised mean differences (*d*) as indices of effect size (Lipsey & Wilson, 2001). Where adjustment for demographic factors led to a substantial change in the effect size associated with risk status (change in $d > 0.30$), stratified analyses were performed to further explore the effect of these variables.

Within-group correlation analyses were conducted to examine the extent to which pituitary volume was associated with cortisol levels, psychosocial stress, and current psychopathology. Pearson's '*r*' correlation analyses were used for normally distributed variables (cortisol AUC values, total daily hassle frequency and distress scales, number of negative life events, and YSR scores) and Spearman's rho '*ρ*' correlation analyses for non-normally distributed variables (negative life event distress scales, and CBCL and PLE scores). Within-group independent samples t-tests were used to examine the relationship between physical punishment and pituitary volume. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 20.

Table 26. Sociodemographic characteristics of participants providing pituitary volume data

	ASz (*n*=30)	FHx (*n*=22)	TD (*n*=32)	Statistics	
				ASz vs. TD	FHx vs. TD
Age (years); mean ± SE	13.1 ± 0.2	13.3 ± 0.2	13.0 ± 0.2	*t*=-0.49 *p*=0.62	*t*=-1.16 *p*=0.25
Time lapse: screening to MRI scan (years); mean ± SE	2.8 ± 0.2	2.6 ± 0.2	2.7 ± 0.1	*t*=-0.77 *p*=0.45	*t*=0.36 *p*=0.72
Pubertal development scale score; mean ± SE [a]	2.4 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	*t*=-0.97 *p*=0.34	*t*=-0.80 *p*=0.43
Weight (kg); mean ± SE	54.8 ± 2.7	51.7 ± 2.6	49.1 ± 1.9	*U*=314.0 *p*=0.07	*U*=265.0 *p*=0.45
BMI (kg/m^2); mean ± SE	20.7 ± 0.6	19.7 ± 0.7	19.7 ± 0.5	*t*=-1.19 *p*=0.24	*t*=-0.01 *p*=0.99
Total intracranial volume (L^3); mean ± SE	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	*t*=-0.20 *p*=0.84	*t*=1.00 *p*=0.32
Sex (male); *n* (%)	20 (67)	11 (50)	15 (47)	χ^2=2.47 *p*=0.13	χ^2=0.05 *p*=1.00
Ethnicity; *n* (%)				***FE*=11.44 *p*=0.008**	***FE*=21.79 *p*<0.001**
White British	6 (20)	2 (9)	18 (56)		
White other	7 (24)	2 (9)	8 (25)		
Black	4 (13)	9 (41)	2 (6)		
Other	13 (43)	9 (41)	4 (13)		
Socioeconomic status; *n* (%) [b]				***FE*=11.43 *p*=0.002**	***FE*=12.82 *p*=0.001**
Higher managerial, administrative, and professional	13 (43)	10 (45)	27 (84)		
Intermediate	11 (37)	3 (14)	4 (13)		
Routine and manual	6 (20)	9 (41)	1 (3)		

Note. Five ASz+FHx cases are included in both groups. BMI: Body Mass Index; *FE*: Fisher's exact. [a] Mean scores in all groups equate to mid-pubertal stage. [b] Socioeconomic status based on caregiver occupation. Missing data: weight (*n*=4); BMI (*n*=4).

6.3 Results

6.3.1 Sample characteristics

Pituitary volumes were obtained for 79 children in total; of these, 25 met ASz criteria only, 17 met FHx criteria only, 5 met both ASz and FHx criteria, and 32 met TD criteria. The five ASz+FHx cases were included in both the ASz and FHx groups, yielding data for 30 ASz and 22 FHx children in total. Sample characteristics are presented by group in Table 26. Neither the ASz nor FHx group were different to the TD group on age, lapse of time between screening and MRI scan, sex, pubertal status, BMI, or total ICV (p>0.10); however, the mean weight of the ASz group was higher than that of the TD group which was significant at the trend level (p=0.07). Relative to the TD group, both the ASz and FHx group were found to differ significantly on ethnicity (p≤0.008) and socioeconomic status (p≤0.001).

6.3.2 Factors associated with pituitary gland volume

Preliminary analyses were conducted in the total sample to identify sociodemographic factors that were associated with pituitary gland volume. Correlation analyses indicated that pituitary volume was not significantly associated with age or total ICV (p>0.10). However, pituitary volume was significantly correlated with weight (ρ=0.26, p=0.02) and pubertal status (r=0.38, p=0.001), and with BMI at the trend level (ρ=0.21, p=0.07). As anticipated, females had significantly larger pituitary gland volumes than males (mean ± SE: 504.8 ± 27.4 vs. 372.8 ± 26.4, respectively; t=-3.47, p=0.001). There was no significant main effect of ethnicity (F[3, 75]=1.17, p=0.33) or socioeconomic status (F[2, 76]=0.05, p=0.96) on pituitary gland volume.

6.3.3 Group differences in pituitary gland volume

Pituitary volumes are presented by group in Figure 12 and Table 27. No differences in pituitary volume were observed when either ASz children (*d*=-0.12, *p*=0.64) or FHx children (*d*=0.04, *p*=0.89) were compared to the TD group. A substantial change in the effect size associated with FHx status was observed after adjustment for demographic factors (*d*=-0.29, *p*=0.46). As sex and pubertal status were found to be significantly associated with pituitary volume in preliminary analyses, stratified analyses were conducted to examine the influence of these variables on the relationship between risk status and pituitary volume. As shown in Table 28, neither ASz nor FHx children differed to the TD group on pituitary volume when males and females were examined separately (*p*>0.80). Similarly, analyses conducted within pubertal strata (pubertal vs. post-pubertal) indicated that there were no group differences in pituitary volume when either ASz or FHx children were compared to the TD group (*p*>0.40).

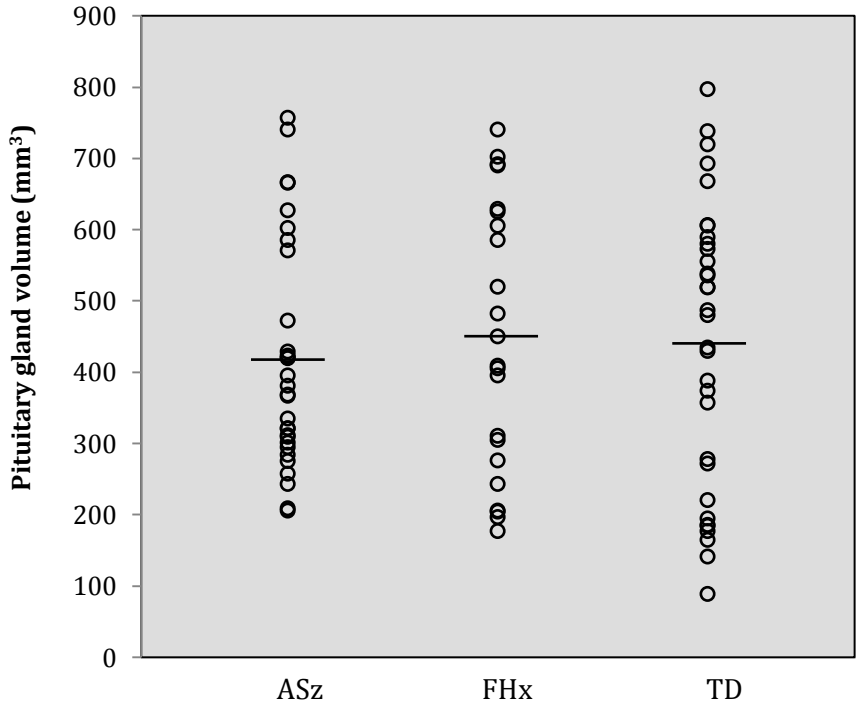

Figure 12. Pituitary volume by group

Note. Horizontal bars indicate group means.

Table 27. Linear regression analyses examining the effect of risk status on pituitary volume

	Descriptive statistics				Statistical analyses							
					ASz vs. TD				FHx vs. TD			
	ASz (*n*=30)	FHx (*n*=22)	TD (*n*=32)	Model [a]	*d*	*B*	(95% CI)	*p*	*d*	*B*	(95% CI)	*p*
Pituitary volume (mm³); mean ± SE	418.7 ± 29.2	447.7 ± 40.4	440.5 ± 35.3	Unadjusted	-0.12	-21.8	(-114.1 – 70.5)	0.64	0.04	7.3	(-101.5 – 116.0)	0.89
				Adjusted	-0.26	-45.0	(-151.4 – 61.4)	0.40	-0.29	-54.3	(-199.4 – 90.8)	0.46

Note. Five ASz+FHx cases are included in both groups. *d*: standardised effect size; *B*: unstandardised regression coefficient. [a] Linear regression analyses without adjustment for potential confounders (unadjusted) and adjusted for sex, ethnicity, socioeconomic status, pubertal developmental scale score, and weight (adjusted).

Table 28. Stratified analyses examining risk status and pituitary volume

	Pituitary volume (mm³); mean ± SE													
	ASz (*n*=30)	FHx (*n*=22)	TD (*n*=32)	Statistics *					ASz (*n*=30)	FHx (*n*=22)	TD (*n*=32)	Statistics *			
				ASz vs. TD		FHx vs. TD						ASz vs. TD		FHx vs. TD	
				d	*p*	*d*	*p*					*d*	*p*	*d*	*p*
Male	375.7 ± 27.8	379.3 ± 54.5	360.0 ± 56.2	0.09	0.80	0.10	0.81	Pubertal	387.5 ± 27.5	369.2 ± 48.8	403.4 ± 46.4	-0.09	0.76	-0.17	0.63
Female	504.5 ± 61.2	516.2 ± 54.3	511.5 ± 38.0	-0.04	0.92	0.03	0.94	Post	521.1 ± 79.3	561.2 ± 51.0	502.3 ± 51.3	0.10	0.84	0.35	0.44

Note. *d*: standardised effect size. * Independent samples t-tests. Pubertal strata based on Tanner stages (Tanner, 1962); pubertal: includes pre-pubertal to late-pubertal (Tanner stage 1–4); post: post-pubertal (Tanner stage 5).

6.3.4 Pituitary volume and salivary cortisol

Consistent with the data presented in Chapter 5, AUCi-CAR values were significantly lower in the FHx group relative to the TD group (p=0.004), reflecting a blunted CAR among FHx children. There were no significant group differences in AUCg-DAY values when either the ASz or FHx group were compared to the TD group (p>0.40). Correlation analyses were conducted to examine the relationship between pituitary volume and cortisol AUC measures (Table 29). There was a strong, positive correlation between AUCi-CAR values and pituitary volume in the ASz group (r=0.52, p=0.005), indicating that a larger pituitary volume was associated with a greater CAR. Positive correlations between AUCi-CAR values and pituitary volume were also observed in the FHx and TD groups, although these associations were weaker and not statistically significant. In contrast, no relationship was observed between pituitary gland volume and AUCg-DAY values in any of the groups (p>0.30).

Table 29. Correlations between pituitary volume and salivary cortisol

	Pituitary gland volume		
	ASz (n=30)	FHx (n=22)	TD (n=32)
AUCi-CAR (cortisol awakening response)	0.52**	0.21	0.17
AUCg-DAY (cortisol levels during the day)	0.04	-0.27	-0.03

Note. * p<0.05; ** p<0.01. Missing data: AUCi-CAR (n=5); AUCg-DAY (n=6).

6.3.5 Pituitary volume and psychosocial stress

Consistent with the pattern of group differences observed in Chapter 4, ASz and FHx children were exposed to a greater number of negative life events than the TD group (p<0.04) and ASz children also reported that they more frequently experienced daily hassles and were more distressed by these hassles than TD children (p<0.004). Correlation analyses were conducted to determine the extent to which pituitary gland volume was associated with negative life events and daily hassles within each group (Table 30). Pituitary volume was not significantly associated with the number of negative life events experienced in any of the three groups. However, among FHx children only, there was a significant negative correlation between pituitary gland volume and current distress relating to negative life events (ρ=-0.42, p=0.05), indicating that smaller pituitary volume was associated with greater distress relating to these events. Pituitary volume was not significantly associated with either daily hassles frequency or distress scores in any group.

Table 30. Correlations between pituitary volume and psychosocial stress

	Pituitary gland volume		
	ASz (n=30)	FHx (n=22)	TD (n=32)
Total number of negative life events	0.20	-0.33	-0.15
Distress at the time of negative life event	0.04	-0.18	-0.11
Current distress related to negative life event	-0.22	-0.42*	-0.05
Frequency of daily hassles	-0.06	0.10	-0.19
Distress related to daily hassles	0.06	0.29	0.17

Note. * $p \leq 0.05$.

6.3.6 Pituitary volume and physical punishment

As previously, physical punishment was more common among ASz (p=0.002) and FHx children (p=0.002) than TD children. Within-group independent samples t-tests were performed to compare pituitary volumes between those who had been exposed to physical punishment and those who had not (Table 31). Among FHx children, those who had experienced physical punishment had significantly smaller pituitary volumes compared to children who had not, equating to a large effect size (d=-1.01, p=0.03). In the ASz and TD groups, however, there was no association between pituitary volume and exposure to physical punishment (p>0.60).

Table 31. Pituitary volume and physical punishment

Pituitary gland volume (mm^3)					
Risk group (n per exposure group [a])	Physical punishment	No physical punishment	d	Statistics *	
ASz (16/14); mean ± SE	405.5 ± 43.9	433.8 ± 38.8	-0.18	t=0.48	p=0.64
FHx (12/10); mean ± SE	367.1 ± 55.5	544.6 ± 44.1	**-1.04**	***t*=2.43**	***p*=0.03**
TD (5/27); mean ± SE	463.7 ± 114.0	436.2 ± 37.3	0.14	t=-0.28	p=0.78

Note. d: standardised effect size. [a] Parentheses indicate number exposed to physical punishment / number not exposed. * Independent samples t-tests.

Sensitivity analyses were conducted to explore the association between pituitary volume and more severe forms of physical punishment. Severe physical punishment was experienced by 8 (27%) ASz children, 8 (36%) FHx children, and none of the TD group; prevalence rates were significantly higher among both ASz (χ^2=9.80, p=0.002) and FHx children (Fisher's exact, p<0.001) compared to TD children. As illustrated in Figure 13, FHx children exposed to severe physical punishment had significantly smaller pituitary volumes compared to FHx children who had not (t=2.58, p=0.02), equating to an even larger effect size (d=-1.11). Again, pituitary volume was not associated with severe physical punishment in the ASz group (t=0.46, p=0.65).

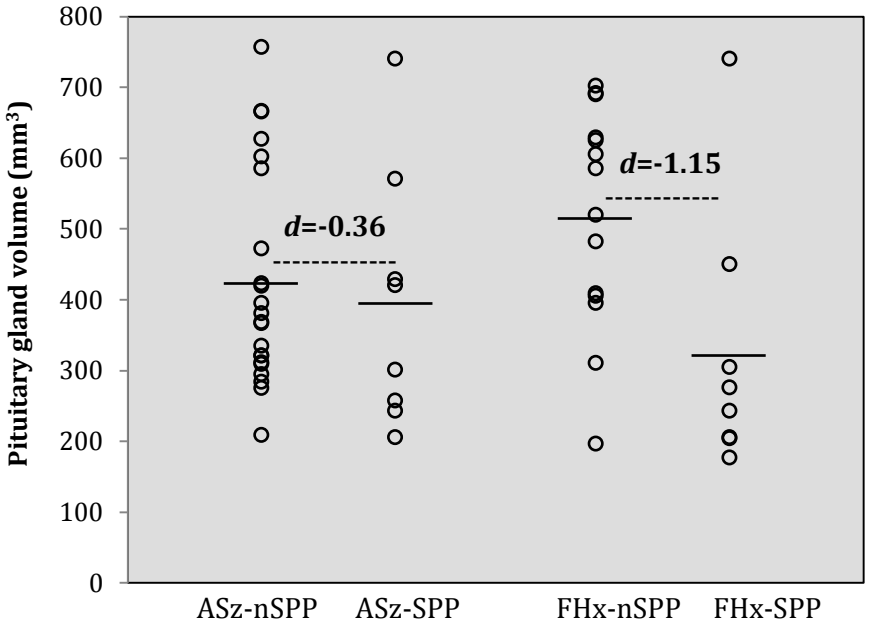

Figure 13. Pituitary volume and severe physical punishment

Note. nSPP: not exposed to severe physical punishment; SPP: exposed to severe physical punishment; *d*: standardised effect size.

6.3.7 Pituitary volume and current psychopathology

Relative to the TD group, ASz children were characterised by significantly higher scores on all psychopathology measures (p<0.03) whilst FHx children obtained higher PLE scores (p=0.05). Within-group correlation analyses conduced to explore the association between pituitary volume and current psychopathology did not identify any significant relationships in any group (Table 32).

Table 32. Correlations between pituitary volume and current psychopathology

	Pituitary gland volume		
	ASz (n=30)	FHx (n=22)	TD (n=32)
YSR Internalising scale (child-report)	0.04	0.05	0.09
YSR Externalising scale (child-report)	-0.26	0.06	-0.16
CBCL Internalising scale (caregiver-report)	-0.10	0.11	-0.09
CBCL Externalising scale (caregiver-report)	-0.08	0.21	-0.28
Psychotic-like experiences (child-report)	-0.03	0.07	0.04

Note. p>0.05 for all correlations. YSR: Youth Self-Report; CBCL: Child Behaviour Checklist.

6.4 Discussion

This is the first study to examine pituitary volume among children at elevated risk for schizophrenia. In contrast to hypotheses, neither children with a family history of illness nor children presenting antecedents of schizophrenia were characterised by abnormal pituitary volume relative to their typically-developing peers. Pituitary volume was not generally associated with salivary cortisol levels, although a positive correlation was observed with the CAR in the ASz group only. Furthermore, among FHx children, pituitary volume was negatively associated with current distress relating to negative life events and with exposure to physical punishment. However, as anticipated, no significant associations between pituitary volume and current psychopathology were observed in any group.

6.4.1 Comparison with previous research

Pituitary volume

The finding that neither FHx nor ASz children were characterised by abnormal pituitary volume relative to the TD group is consistent with some previous studies of individuals at elevated risk for schizophrenia, but not all. Mondelli and colleagues observed significantly larger pituitary volumes among adult relatives of patients with schizophrenia (Mondelli et al., 2008), yet a subsequent study found no differences between adult relatives and controls (Habets et al., 2012). Relatives in the former study were often the parents of patients and were older than those examined in the study by Habets and colleagues (mean age: 49.7 vs. 28.3 years, respectively). Thus, age-related changes in pituitary volume may have contributed to the differences across studies. Inconsistent findings have also been reported by studies of young adults with SPD. Takahashi et al. (2009) observed significantly larger pituitary volumes in individuals with SPD relative to healthy controls, yet a further study observed smaller pituitary volumes among males, but not females, with SPD (Romo-

Nava et al., 2013). The contrast in findings is likely to reflect differences in sample characteristics. Whilst the SPD groups in both studies were of a similar age (mean age: 25.0 vs. 29.4 years, respectively), individuals with SPD in the former study were psychiatric patients, the majority of whom were receiving antipsychotic medication, however, individuals in the latter study were recruited from the community and were all medication-naive. Thus, differences in age, illness severity, and medication status may explain why the current findings do not support previous studies reporting abnormal pituitary volume among high-risk individuals. In contrast, two studies of UHR youth observed no differences in pituitary volume between at-risk youth and healthy controls (Garner et al., 2005; Büschlen et al., 2011), although both studies observed pituitary volume increases among UHR youth who later transitioned to psychosis compared to both UHR youth who did not transition and healthy controls. These findings suggest that, among UHR youth at least, pituitary volume abnormalities may be specific to those who later go on to develop psychosis. This may also account for the lack of pituitary volume abnormalities observed among ASz and FHx children (i.e., as these groups are likely to include both individuals who will develop illness and those who will not).

Pituitary volume and cortisol

The current study observed that pituitary gland volumes were not significantly associated with diurnal cortisol levels, although a positive association was observed with the CAR among ASz children only. The relationship between pituitary volume and cortisol levels has scarcely been examined among individuals at elevated risk for schizophrenia, or indeed among healthy individuals. Previous studies of individuals with a family history of psychosis (Habets et al., 2012) and youth at UHR (Thompson et al., 2007) similarly observed no relationship between pituitary volume and cortisol levels during the day. However, a recent study of healthy adolescents observed

positive associations between pituitary volume and the CAR, and negative associations between pituitary volume and diurnal cortisol levels (Kaess et al., 2013); though both relationships were present only when boys were examined in isolation. Thus, it is possible that the significant correlation identified between pituitary volume and the CAR among only ASz children may reflect the fact that a larger proportion of this group were male compared to the FHx and TD groups.

Pituitary volume and psychosocial stress

Among FHx children, a significant negative correlation was observed between pituitary volume and current distress relating to negative life events. Only one previous study of high-risk individuals has examined the relationship between pituitary volume and psychosocial stress; Habets and colleagues (2012) reported that pituitary volume among patients with psychosis, and to a lesser extent their adult siblings and healthy controls, was *positively* associated with emotional stress reactivity (i.e., the increase in negative affect in response to daily stressful experiences). Whilst these findings appear to contrast with those of the current study, of note, pituitary volume was positively associated with distress relating to daily hassles among FHx, ASz, and TD children, although none of these effects reached statistical significance. The conflicting pattern of findings may reflect the type of stressor examined; whilst greater distress relating to major traumatic events appears to be associated with smaller pituitary volume (at least among children with a family history of schizophrenia), emotional reactivity to minor stressors may be related to larger pituitary volume.

The current study also observed that FHx children who had experienced physical punishment had significantly smaller pituitary volumes than those who had not, equating to a large effect size. The same pattern was observed when more severe forms of physical punishment were examined in isolation, with an even larger

magnitude of effect. This finding is consistent with a previous study of adolescents with borderline personality disorder (BPD) which found that whilst there were no differences in pituitary volume between adolescents with BPD and healthy controls, among those with BPD, those who had been exposed to childhood maltreatment (most commonly, physical abuse) were characterised by smaller pituitary volumes compared to those who had not experienced maltreatment (Garner et al., 2007). The current findings suggest that the relationship between pituitary volume and childhood maltreatment may extend to less severe forms of physically abusive parenting.

Pituitary volume and current psychopathology

As hypothesised, pituitary volumes were not significantly correlated with PLEs in any group or with internalising or externalising symptoms. Previous studies of UHR youth (Garner et al., 2005) and patients with SPD (Takahashi et al., 2009) have also reported no relationship between pituitary volume and psychotic symptoms. Furthermore, a study of adult relatives of patients with schizophrenia reported that pituitary volume was not significantly correlated with schizotypal symptoms (Mondelli et al., 2008). Consistent with these findings, a study of healthy adolescents observed no relationship between current psychopathology (total CBCL scores) and pituitary volume (Zipursky et al., 2011). Thus, the current study adds to existing literature showing that pituitary volume is unrelated to current symptomology.

6.4.2 Potential mechanisms

Pituitary volume abnormalities in psychosis

One possible explanation for why ASz and FHx children were not characterised by abnormal pituitary volume relative to the TD group is that this neuroanatomical feature is associated with more severe clinical presentation. Enlarged pituitary volume has been observed among patients who have recently experienced their first

psychotic episode (Pariante et al., 2004; Pariante et al., 2005; Büschlen et al., 2011; Takahashi et al., 2011) and individuals with SPD who exhibit symptoms of sufficient severity to warrant antipsychotic treatment (Takahashi et al., 2009). Furthermore, two studies reported that youth at UHR who developed psychosis within 1-4 years had significantly larger pituitary volumes compared to UHR who did not (Garner et al., 2005; Büschlen et al., 2011). Thus, pituitary volume abnormalities may emerge in response to the onset of acute illness. However, the fact that enlarged pituitary volumes have also been observed among relatives of patients with schizophrenia who are not acutely unwell (Mondelli et al., 2008), suggests that genetic liability may play some role in the expression of pituitary volume abnormalities among individuals with psychosis. Whilst the current findings and those of a previous study of young adult relatives (Habets et al., 2012) do not support this hypothesis, it is possible that the larger pituitary volumes characterising older adult relatives reflects a genetic predisposition to HPA axis hyperactivity that emerges with increasing age following accumulated life stress.

Relationship between physical punishment and pituitary volume

A novel finding was that FHx children who were exposed to physical punishment had significantly smaller pituitary volumes compared to those who had not. Whilst a similar relationship between childhood maltreatment and pituitary volume has been reported among adolescents with BPD (Garner et al., 2007), the same pattern of findings was not observed among ASz and TD children. Furthermore, this did not appear to be due to the fact that FHx children had simply experienced more severe forms of physical punishment. One possible explanation for the findings is that FHx children may have been more distressed by experiences of physical punishment, which may in turn have had a greater effect on pituitary volume in this group. Indeed, as shown in Chapter 4, physical punishment was positively associated with

internalising symptoms and PLEs among FHx children, but not in ASz or TD children. Consistent with this hypothesis, current distress relating to negative life events was also negatively associated with pituitary volume among FHx children only.

It has been suggested that elevated cortisol levels, resulting from stress-induced HPA axis hyperactivity, may have an inhibitory action on pituitary corticotrophs (i.e., the cells within the anterior pituitary that are responsible for ACTH production), eventually leading to pituitary volume reduction (Sassi et al., 2001). Thus, whilst stressful experiences might have been associated with increased cortisol levels and enlarged pituitary volume at the time that they occurred, enduring distress relating to these experiences among FHx children might feasibly result in pituitary volume reduction. However, as cortisol levels were only assessed at one time-point, not necessarily coinciding with stress exposure, this explanation is speculative. Alternatively, smaller pituitary volumes may predispose some FHx children to experience greater distress in response to negative life events and physical punishment. For example, a longitudinal study of adolescents observed no relationship between pituitary volume and psychopathology at baseline, but found that larger pituitary volumes predicted an increase in internalising symptoms over time (Zipursky et al., 2011). Unfortunately, the cross-sectional nature of these analyses means that it is not possible to disentangle the temporal relationship between experiences of psychosocial stress and pituitary gland volume.

6.4.3 Methodological considerations

Chapter 8 provides a more detailed discussion of the methodological issues that influence the overall study. Specific issues that are relevant to the data presented in this chapter are described in this section. One potential limitation relates to the characteristics of children who were not able to complete an MRI scan. Given that pituitary volume data was obtained for only 71% of children who participated in the

24-month assessment phase, it is possible that children who were not scanned were characterised by higher levels of stress reactivity (and perhaps pituitary volume abnormalities) which may have precluded the ability to detect group differences in pituitary volume. However, within-group analyses confirmed that children who provided pituitary volume data did not differ from those who did not on psychopathology scores at screening (Chapter 3). A further limitation relates to the fact that whilst biological indices of HPA axis function were collected during the same assessment phase, these measures were not always completed on the same day. This might explain why cortisol levels were not associated with pituitary gland volume across the groups.

6.4.4 Conclusions

The current study indicates that children at elevated risk for schizophrenia are not characterised by abnormal pituitary gland volume compared to their typically-developing peers. These findings are consistent with the work presented in Chapter 5, and imply that the biological markers of HPA axis hyperactivity (i.e., elevated cortisol levels during the day and enlarged pituitary volume) that have been observed in older samples of high-risk individuals may emerge later, more proximally to disease onset. Among FHx children only, pituitary volume was negatively associated with current distress relating to negative life events and exposure to physical punishment. This may reflect a genetically-mediated effect which predisposes FHx children to experience more distress in response to psychosocial stressors. Alternatively, these experiences may have caused an initial increase in HPA axis activity, which due to enduring distress, subsequently leads to a reduction in pituitary volume. Longitudinal studies of children at elevated risk for developing schizophrenia are needed to establish the temporal relationship between psychosocial stress exposure, cortisol levels, and pituitary volume abnormalities.

CHAPTER 7 Exploratory associations of neurocognitive function with psychosocial stress and cortisol

7.1 Introduction

Neurocognitive dysfunction is a core feature of schizophrenia. Relative to healthy individuals, patients with schizophrenia are characterised by moderate-to-large deficits across multiple neurocognitive domains, with the most severe impairments observed in memory and executive function (Reichenberg & Harvey, 2007). Similar impairments, albeit smaller in magnitude, have also been observed among youth with a family history of schizophrenia (Agnew-Blais & Seidman, 2013) and UHR youth (Fusar-Poli et al., 2012b). Whist it is likely that these neurocognitive deficits are to a large extent driven by genetic factors (Kahn & Keefe, 2013), there is some evidence to suggest that psychosocial stress exposure and abnormal HPA axis function may contribute to some of the neurocognitive features of schizophrenia.

Animal studies indicate that stress-induced HPA axis dysfunction can lead to structural abnormalities in the hippocampus and medial prefrontal cortex, regions which play a crucial role in mediating HPA axis function (Herman et al., 2005). Specifically, studies of rodents have observed that chronic stress and persistently-elevated glucocorticoid levels can cause hippocampal cell damage (Sapolsky, 2000), and that behavioural stress is associated with structural changes in the medial prefrontal cortex (Cerqueira et al., 2008). Thus, the deficits in memory and executive function that have been consistently observed among individuals with schizophrenia, may, at least in part, be due to the adverse effects of psychosocial stress and cortisol on the brain regions that support these neurocognitive functions. In support of this notion, elevated cortisol levels have been associated with poorer performance on tests of memory and executive function among individuals with schizophrenia

(Walder et al., 2000), and a more blunted CAR has been found to correlate with greater deficits in verbal memory in first-episode patients (Aas et al., 2011b). However, the extent to which the observed associations between these neurocognitive functions and cortisol are triggered by psychosocial stress exposure is currently unclear. Whilst some studies of individuals with schizophrenia have reported greater impairments in memory and executive function among those exposed to childhood maltreatment (Lysaker et al., 2001; Shannon et al., 2011), other studies have observed no association between psychosocial stress exposure (including childhood maltreatment) and these neurocognitive functions (Schenkel et al., 2005; Aas et al., 2011b; Sideli et al., in press). Further investigations employing concurrent measures of neurocognitive function, psychosocial stress, and cortisol levels are therefore required to elucidate this relationship. Moreover, it is not yet known whether the neurocognitive deficits that characterise individuals at elevated risk for schizophrenia are also associated with experiences of psychosocial stress and HPA axis dysfunction.

Chapter aims

This chapter aimed to explore the extent to which neurocognitive function is associated with experiences of psychosocial stress and cortisol levels in children at elevated risk for schizophrenia who present multiple antecedents of the disorder (ASz) or a family history of illness (FHx), and typically-developing (TD) children.

Hypotheses

4a. Experiences of psychosocial stress (exposure and reactivity) will be negatively associated with neurocognitive function in ASz and FHx children.

4b. Among ASz and FHx children, more abnormal cortisol levels will be associated with poorer neurocognitive function.

7.2 Methods

7.2.1 Participants and procedure

As described in Chapter 3, ASz and TD children were recruited using a novel community-screening procedure (Laurens et al., 2007; Laurens et al., 2011), and FHx children were identified either via the caregiver screening questionnaire or as relatives of patients with schizophrenia or schizoaffective disorder. Children meeting ASz, FHx, or TD criteria were invited to participate in a longitudinal study of child development. This chapter examines data from assessments of neurocognitive function, psychosocial stress, and salivary cortisol obtained at the 24-month assessment phase.

7.2.2 Neurocognitive assessments

Participants completed a battery of neurocognitive assessments with a trained researcher within six months of completing psychosocial stress measures and salivary cortisol collection (median lapse of time between neurocognitive tests and completion of psychosocial stress measures and salivary cortisol assessments: ± 0.5 months for both). Selected subtests from the Wide Range Assessment of Memory and Learning 2nd Edition [WRAML2 (Sheslow & Adams, 2003)] and the Delis–Kaplan Executive Function System [D-KEFS: (Delis et al., 2001)] were used to assess memory (verbal memory, visual memory, attention and working memory) and executive function (verbal fluency, inhibition, and planning ability). These neurocognitive domains were examined as indices of functioning in the hippocampus and medial prefrontal cortex (Antonova et al., 2004; Reichenberg & Harvey, 2007; Euston et al., 2012; Orellana & Slachevsky, 2013), which are known to mediate HPA axis function (Herman et al., 2005). Subtests are described in Table 33; standardised scores were derived for each subtest using age-adjusted norms.

Table 33. Neurocognitive measures

Domain	Subtest	Test description
WRAML2 subtests		
Verbal memory	Story memory [a]	Immediate recall of two short stories presented orally
	Verbal learning [a]	Immediate free recall of a list of words presented orally
Visual memory	Design memory [b]	Drawing five visually-presented geometric designs
	Picture memory [b]	Identifying differences between four similar picture pairs
Attention and working memory	Number-letter	Repeating strings of numbers and letters presented orally
	Verbal working memory	Immediate recall of word lists by category (animal vs. non-animal)
D-KEFS subtests		
Verbal fluency	Letter fluency	Generating words beginning with F, A, and S within 60 seconds
	Category fluency	Generating items from categories (animals and boys names) within 60 seconds
	Category switching	Generating words from alternate categories (fruit and furniture) within 60 seconds
Inhibition/attention	CWIT – Inhibition condition	Naming the ink colour of colour-words presented in contrasting coloured ink (Stroop, 1935)
	CWIT – Inhibition/switching	Alternating between the above instruction or naming the colour-word (ignoring ink colour)
Planning	Towers test (total achievement)	Building towers using one to five disks in the fewest possible moves

Note. WRAML2: Wide Range Assessment of Memory and Learning 2nd Edition (Sheslow & Adams, 2003); D-KEFS: Delis–Kaplan Executive Function System (Delis et al., 2001); CWIT: colour-word interference test. [a] Standardised scores on the story memory and verbal learning subtests summed to derive a verbal memory index score; [b] standardised scores on the design memory and picture memory subtests summed to derive a visual memory index score.

7.2.3 Psychosocial stress

Psychosocial stress measures were described in detail in Chapter 4. In brief, children completed questionnaires assessing negative life events and school-related daily hassles (Heubeck & O'Sullivan, 1998). Physical punishment was assessed using the corporal punishment scale of the Alabama Parenting Questionnaire [APQ (Shelton et al., 1996)], completed by the child at the 24-month assessment phase.

7.2.4 Salivary cortisol

The saliva collection protocol was described in detail in Chapter 5. Briefly, participants collected six saliva samples throughout the day on two consecutive days: upon awakening, at 15, 30, and 60 min after awakening, and at 12:00 pm and 20:00 pm. Cortisol data obtained at individual time-points were summarised using two area under the curve (AUC) computations (Pruessner et al., 2003); (i) AUC with respect to the increase in cortisol levels following awakening (AUCi-CAR), and (ii) AUC with respect to ground of cortisol levels during the day (AUCg-DAY).

7.2.5 Statistical analyses

As neurocognitive test scores were approximately normally distributed, independent samples t-tests were used to examine group differences (ASz vs. TD and FHx vs. TD) on these measures. As previously, children meeting both ASz and FHx criteria were retained in both groups, thus, ASz and FHx groups were not compared directly. Within-group correlation analyses were conducted to examine associations between neurocognitive test scores and psychosocial stress measures and cortisol AUC values. Pearson's '*r*' correlation analyses were used for normally distributed variables (number of negative life events, daily hassles scales, and cortisol AUC values) and Spearman's rho '*ρ*' correlations for non-normally distributed variables (negative life event distress scales and APQ physical punishment scores). All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 20.

7.3 Results

7.3.1 Sample characteristics

As described previously, 95 children (ASz=29, FHx=19, ASz+FHx=5, TD=42) completed psychosocial stress measures and 91 children (ASz=29, FHx=18, ASz+FHx=4, TD=40) completed the saliva collection protocol; sociodemographic characteristics of these samples are provided in Chapters 4 and 5, respectively. In brief, in both the psychosocial stress sample and the salivary cortisol sample, there were no significant group differences (i.e., ASz vs. TD or FHx vs. TD) in age, pubertal status, BMI, tobacco use, or lapse of time between antecedent screening and completion of psychosocial stress measures and salivary cortisol collection (p>0.20). Additionally, the groups did not differ on the lapse of time between neurocognitive testing and psychosocial stress measure completion or saliva collection (p>0.20). In both samples, ASz children were more likely to be male than TD children (p<0.04), and the ASz and FHx groups were both found to differ significantly from TD children on ethnicity (p≤0.05) and socioeconomic status (p≤0.006).

7.3.2 Group differences in neurocognitive function

All participants who provided psychosocial stress data and/or salivary cortisol data completed tests of memory (WRAML2) and executive function (D-KEFS) yielding neurocognitive data for 99 children in total (87 children provided both psychosocial stress and salivary cortisol data, 8 provided psychosocial stress data only, and 4 provided salivary cortisol data only). Neurocognitive data are shown for the total sample of children by risk group in Table 34. Relative to TD children, the ASz group showed poorer performance on the WRAML2 number-letter subset (t=2.39, p=0.02) and the D-KEFS verbal fluency – category switching subtest (t=2.20, p=0.03). FHx children obtained lower scores on the D-KEFS colour-word interference – inhibition subtest compared to TD children (t=2.14, p=0.02).

Table 34. Group differences in neurocognitive function

	ASz (*n*=35)	FHx (*n*=25)	TD (*n*=44)	Statistics	
				ASz vs. TD	FHx vs. TD
Memory (WRAML2); mean ± SE					
Number-letter	11.3 ± 0.5	11.7 ± 0.7	13.0 ± 0.4	***t*=2.39 *p*=0.02**	*t*=1.58 *p*=0.12
Verbal memory	22.1 ± 0.7	21.1 ± 0.8	23.2 ± 0.8	*t*=0.94 *p*=0.35	*t*=1.68 *p*=0.10
Visual memory	16.1 ± 0.8	16.6 ± 1.0	17.8 ± 0.6	*t*=1.70 *p*=0.09	*t*=1.07 *p*=0.29
Working memory	9.5 ± 0.4	9.9 ± 0.5	10.5 ± 0.4	*t*=1.80 *p*=0.08	*t*=1.00 *p*=0.32
Executive functioning (D-KEFS); mean ± SE					
Verbal fluency – Letter fluency	10.5 ± 0.5	10.6 ± 0.6	10.9 ± 0.4	*t*=0.67 *p*=0.50	*t*=0.37 *p*=0.71
Verbal fluency – Category fluency	12.4 ± 0.6	11.5 ± 0.7	12.5 ± 0.5	*t*=0.23 *p*=0.82	*t*=1.29 *p*=0.20
Verbal fluency – Category switching	9.8 ± 0.5	10.8 ± 0.7	11.5 ± 0.6	***t*=2.20 *p*=0.03**	*t*=0.76 *p*=0.45
Colour-word interference – Inhibition	11.0 ± 0.4	10.4 ± 0.5	11.7 ± 0.3	*t*=1.38 *p*=0.17	***t*=2.14 *p*=0.04**
Colour-word interference – Inhibition / switching	10.7 ± 0.3	10.5 ± 0.5	11.0 ± 0.4	*t*=0.64 *p*=0.53	*t*=0.89 *p*=0.38
Tower test – Total achievement score	11.1 ± 0.3	11.4 ± 0.3	11.4 ± 0.3	*t*=0.78 *p*=0.44	*t*=-0.05 *p*=0.96

Note. Five ASz+FHx cases are included in both groups. WRAML2: Wide Range Assessment of Memory and Learning 2nd Edition; D-KEFS: Delis–Kaplan Executive Function System. Possible score ranges for all tests: 0–20, except for the verbal memory index and the visual memory index where the score range is 0–40.

7.3.3 Neurocognitive function and psychosocial stress

Correlations between neurocognitive function and psychosocial stress measures (exposure and distress relating to negative life events, total daily hassles frequency and distress scales, and APQ physical punishment scale scores) are presented in Table 35. Among ASz children, the number of negative life events experienced was positively correlated with scores on the D-KEFS verbal fluency – category switching (r=0.44, p=0.008) and colour-word interference – inhibition switching subtests (r=0.35, p=0.05). Additionally, previous distress relating to negative life events was positively associated with scores on the D-KEFS colour-word interference – inhibition switching subtest in the ASz group (ρ=0.35, p=0.04). Among TD children, the number of negative life events experienced was moderately positively correlated with WRAML2 verbal memory performance (ρ=0.35, p=0.02), whilst previous distress relating to negative life events was positively associated with WRAML2 visual memory performance (ρ=0.33, p=0.03). In contrast, none of the neurocognitive measures were associated with the number of life events experienced or with distress relating to these events (either previous or current) in FHx children.

Total daily hassles frequency scores were moderately positively correlated with D-KEFS verbal fluency – letter fluency (r=0.37, p=0.03) and category fluency subtest scores (r=0.35, p=0.05) among ASz children, and were also positively correlated with performance on the WRAML2 verbal memory index in the FHx group (r=0.41, p=0.05). Scores on the daily hassles frequency and distress scales were not significantly associated with any measure of neurocognitive functioning in the TD group. Finally, among ASz children only, child-reported scores on the APQ physical punishment scale were positively correlated with performance on the D-KEFS verbal fluency – letter fluency subtest (ρ=0.37, p=0.03).

Table 35. Correlations between neurocognitive function and psychosocial stress

	ASz (*n*=34)						FHx (*n*=24)						TD (*n*=42)					
	NLE No	NLE prev	NLE curr	DH freq	DH dist	PP score	NLE No	NLE prev	NLE curr	DH freq	DH dist	PP score	NLE No	NLE prev	NLE curr	DH freq	DH dist	PP score
Memory (WRAML2)																		
Number-letter	0.09	0.24	0.26	-0.03	0.21	0.09	-0.08	0.29	0.35	0.16	0.00	0.04	0.25	0.08	-0.06	-0.08	-0.15	-0.01
Verbal memory	-0.09	0.13	-0.01	-0.06	-0.03	-0.08	-0.14	0.20	0.37	0.41*	-0.10	0.00	0.35*	0.27	0.11	0.17	-0.19	-0.28
Visual memory	0.15	0.16	-0.05	0.15	-0.16	0.26	0.33	0.16	0.06	0.16	0.16	-0.08	0.22	0.33*	0.21	-0.13	-0.27	-0.22
Working memory	0.08	0.10	0.04	-0.11	0.19	0.04	0.01	0.19	0.35	0.15	0.12	0.08	0.08	0.14	-0.01	0.02	-0.27	0.18
Exec Func (D-KEFS)																		
Letter fluency	0.08	0.09	0.22	0.37*	0.15	0.37*	0.28	0.15	0.17	0.31	0.25	0.23	0.05	-0.04	-0.10	0.27	0.24	0.15
Category fluency	0.24	0.15	0.14	0.35*	-0.13	0.00	0.24	0.10	0.19	0.36	0.41	0.22	0.07	0.06	0.02	0.11	-0.12	-0.14
Category switching	0.44**	0.31	0.11	0.05	-0.10	0.00	-0.10	0.07	0.06	0.02	0.03	-0.33	0.21	0.16	0.07	-0.01	-0.09	-0.09
Inhibition	0.27	0.26	0.01	-0.02	-0.12	-0.27	0.00	0.13	0.19	0.15	0.05	-0.20	0.22	0.01	-0.10	-0.02	-0.26	-0.13
Inhibition/switching	0.35*	0.35*	0.22	-0.03	0.12	-0.13	0.14	0.24	0.20	0.14	0.26	-0.05	0.20	0.14	0.14	-0.10	0.06	-0.22
Towers test	0.28	-0.06	0.08	0.00	0.10	0.30	0.31	-0.09	0.10	0.02	0.03	0.03	0.04	0.14	0.07	0.24	0.08	0.16

Note. * $p<0.05$; ** $p<0.01$; WRAML2: Wide Range Assessment of Memory and Learning 2nd Edition; Exec Func: executive function; D-KEFS: Delis–Kaplan Executive Function System; NLE No: total number of negative life events; NLE prev: previous distress relating to negative life events; NLE curr: current distress relating to negative life events; DH freq: daily hassles frequency scores; DH dist: daily hassle distress scale; PP score: physical punishment scale score.

7.3.4 Neurocognitive function and cortisol

Correlation analyses were performed to examine the relationship between neurocognitive function and cortisol AUC measures (Table 36). AUCi-CAR values were *positively* correlated with scores on the D-KEFS verbal fluency – letter fluency subtest among ASz children (r=0.41, p=0.02) and with WRAML2 verbal memory index scores in the FHx group (r=0.46, p=0.04), but were not significantly associated with any neurocognitive measure in the TD group. In contrast, among FHx children, AUCg-DAY values were *negatively* correlated with scores on the WRAML2 verbal memory index (r=-0.47, p=0.05), WRAML2 number-letter subtest (r=-0.62, p=0.008), and the D-KEFS Towers test (r=-0.55, p=0.02). Relationships between verbal memory and cortisol AUC values are further illustrated in Figure 14.

Table 36. Correlations between neurocognitive function and cortisol

	ASz (n=33)		FHx (n=22)		TD (n=40)	
	AUCi-CAR	AUCg-DAY	AUCi-CAR	AUCg-DAY	AUCi-CAR	AUCg-DAY
Memory (WRAML2)						
Number-letter	0.19	-0.01	0.29	-0.62**	-0.22	0.16
Verbal memory	0.04	0.17	0.46*	-0.47*	0.03	-0.12
Visual memory	-0.05	0.22	0.05	-0.31	0.24	-0.02
Working memory	0.09	0.26	0.08	-0.36	-0.09	-0.06
Exec Func (D-KEFS)						
Letter fluency	0.41*	-0.30	0.23	-0.43	-0.14	0.27
Category fluency	0.22	0.06	0.02	-0.30	0.18	0.15
Category switching	-0.14	0.16	0.22	0.05	0.06	0.11
Inhibition	-0.16	0.09	0.27	-0.06	0.01	0.03
Inhibition/switching	-0.06	-0.05	0.27	0.02	-0.03	-0.04
Towers test	0.22	-0.27	0.18	-0.55*	0.10	0.17

Note. * p<0.05; ** p<0.01. WRAML2: Wide Range Assessment of Memory and Learning 2nd Edition; Exec Func: executive function; D-KEFS: Delis–Kaplan Executive Function System.

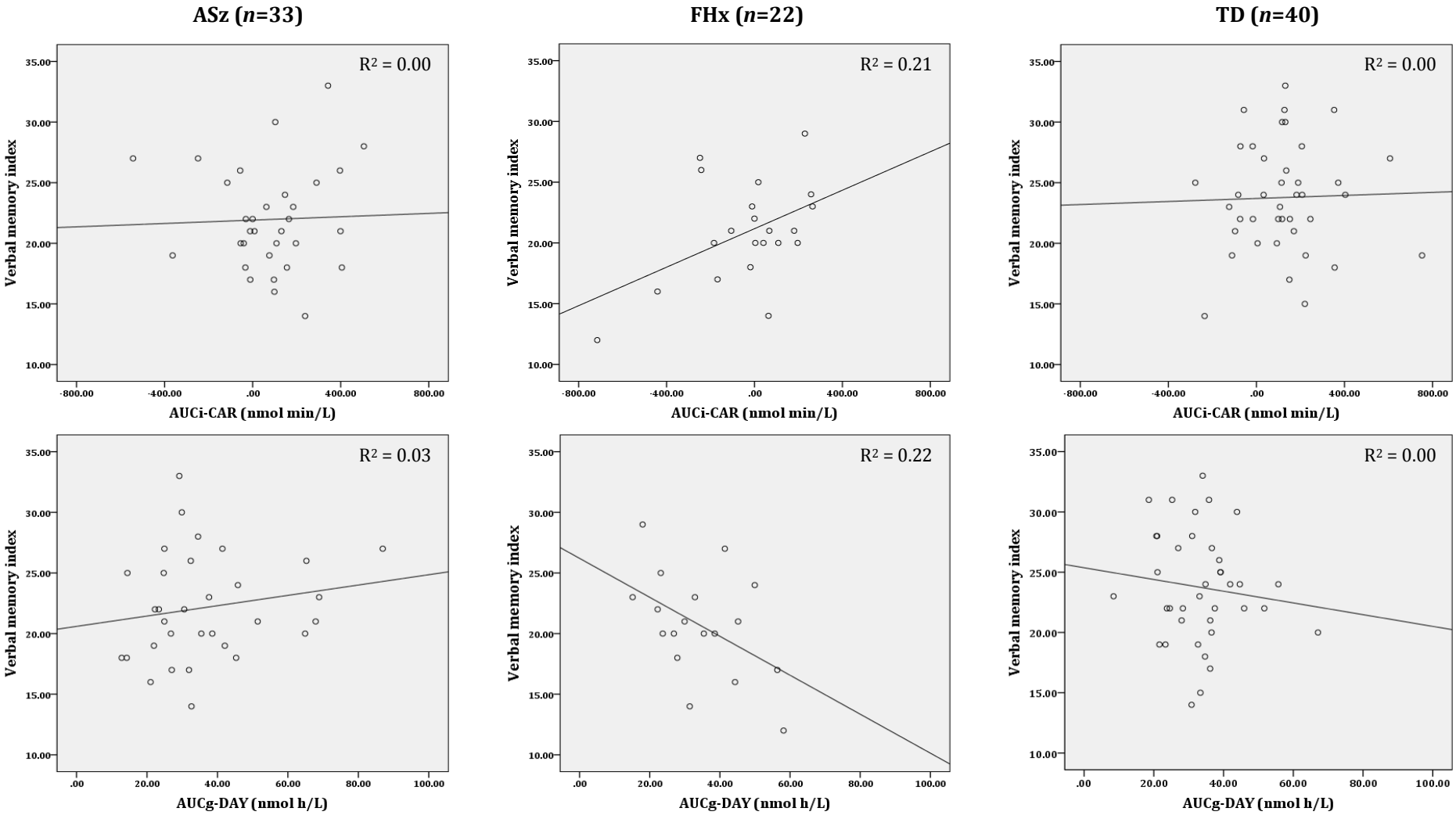

Figure 14. Scatterplots illustrating the relationship between verbal memory performance and cortisol levels

7.4 Discussion

This chapter explored the extent to which neurocognitive function among children at elevated risk for schizophrenia is associated with experiences of psychosocial stress and salivary cortisol. As hypothesised, among FHx and ASz children, more abnormal HPA axis function (that is, higher diurnal cortisol levels and a more blunted CAR) was associated with poorer performance on tests of verbal memory and executive function. Neurocognitive function was not associated with cortisol levels in the TD group. In contrast to predictions, experiences of psychosocial stress (both higher levels of exposure and greater distress in relation to these exposures) were associated with better performance on neurocognitive measures in all three groups.

7.4.1 Comparison with previous research

Neurocognitive function and psychosocial stress

The current study observed that among ASz children, better performance on executive function tasks was associated with higher levels of psychosocial stress exposure (negative life events, daily hassles, and physical punishment). Furthermore, verbal memory was positively associated with exposure to daily hassles and negative life events in FHx and TD children, respectively. These findings are in contrast with previous studies which have reported that adults with schizophrenia who have been exposed to severe forms of psychosocial stress, namely, childhood maltreatment, show poorer performance on tests of working memory, episodic memory, and executive function (Lysaker et al., 2001; Shannon et al., 2011). However, other studies have observed no association between psychosocial stress exposure (including childhood maltreatment) and these neurocognitive deficits among individuals with psychosis (Schenkel et al., 2005; Aas et al., 2011b; Sideli et al., in press). Indeed, Aas and colleagues reported that psychosocial stress exposure (childhood trauma, stressful life events, and perceived stress) was associated with

poorer neurocognitive function among healthy individuals but not among individuals with first-episode psychosis (Aas et al., 2011b), a finding that was replicated in a larger investigation of first-episode patients recruited to this study (Sideli et al., in press). A similar association was observed in a study of individuals with established schizophrenia (McCabe et al., 2012), in which a negative relationship was reported between exposure to childhood adversity and IQ among healthy controls but not among patients. However, in a different sample of first-episode patients, Aas and colleagues observed that childhood trauma was correlated with poorer neurocognitive performance, but only among male patients and those with affective psychosis (Aas et al., 2011a). Thus, previous studies investigating the relationship between psychosocial stress exposure and neurocognitive function among individuals with psychosis have yielded inconsistent findings. This may relate to differences in the specific psychosocial stressors examined (discussed below) and/or variability in patient characteristics across studies.

Among ASz and TD children, the level of distress experienced in relation to negative life events at the time of the event was positively associated with executive function (specifically, the inhibition/switching condition of the Stroop colour-word interference test) and visual memory, respectively. Although unexpected, these findings are, in fact, consistent with two previous studies which examined the relationship between neurocognitive function and stress reactivity in individuals with psychosis using the ESM technique (Myin-Germeys et al., 2002; Morrens et al., 2007). In both studies, greater reactivity to stressful events throughout the day (i.e., greater decreases in positive affect and greater increases in negative affect) was associated with better performance on a verbal memory test and the Stroop colour-word interference test.

Neurocognitive function and cortisol

Exploratory analyses were also conducted to examine the relationship between neurocognitive function and cortisol levels. These analyses demonstrated that neurocognitive deficits are associated with more abnormal cortisol levels in children at elevated risk for schizophrenia. Specifically, a greater blunting of the CAR was associated with poorer verbal memory and letter fluency among FHx and ASz children, respectively; moreover, in the FHx group only, higher diurnal cortisol was correlated with poorer performance on the number-letter subtest and Towers test, and lower verbal memory index scores. In contrast, cortisol levels were not associated with neurocognitive performance among TD children. Whilst this is the first study to have examined the relationship between neurocognitive deficits and HPA axis function in a sample of high-risk individuals, the current findings are consistent with studies of adults with established illness. A previous study examining individuals with chronic schizophrenia, patients with other psychiatric disorders, and healthy controls, found that higher cortisol levels during the day were correlated with poorer performance on tasks of memory and executive function when associations were examined across the total sample (Walder et al., 2000). A subsequent study observed that a more blunted CAR was associated with deficits in verbal memory and processing speed among individuals with first-episode psychosis but not healthy controls (Aas et al., 2011a), although none of the neurocognitive measures were associated with cortisol levels during the day. The reason for this slight difference in findings is unclear, but again, may relate to differences in patient characteristics and/or the neurocognitive tests employed. Nonetheless, these studies, and the current investigation, concur in showing that more abnormal cortisol levels are associated with poorer performance on specific measures of neurocognitive function in individuals with schizophrenia and those at elevated risk for the disorder.

7.4.2 Potential mechanisms

Relationship between psychosocial stress and neurocognitive function

The finding that experiences of psychosocial stress (both higher levels of exposure and greater distress in relation to these exposures) were associated with better performance on tests of executive function and memory was unexpected. Indeed, trauma exposure has been consistently associated with poorer performance on tests of memory and executive function in children and adults without schizophrenia (Porter et al., 2005; Gould et al., 2012; Spann et al., 2012). Previous studies indicate that the extent to which experiences of psychosocial stress impair or enhance neurocognitive function may depend on the specific type of stress exposure. A study of older adults reported that whilst some psychosocial stressors (namely, serious injury or illness in a friend and greater distress in relation to this experience) were associated with better performance on memory and executive function tasks, other stressful experiences (i.e., recent financial difficulties and distress relating to being a victim of crime) were associated with poorer neurocognitive function (Rosnick et al., 2007). Similarly, a subsequent study of older adults reported that the death of a relative was associated with poorer neurocognitive function whilst illness in a partner or relative was positively correlated with neurocognitive performance (Comijs et al., 2011). It has been hypothesised that the effect of stressful life events on neurocognitive function may be mediated by long-term changes in arousal (Rosnick et al., 2007; Feeney et al., 2013). Thus, whilst some psychosocial stressors may increase arousal to a degree that allows the individual to perform optimally, other stressors may cause hyper- or hypo-arousal which impairs neurocognitive abilities (Rosnick et al., 2007). Thus, the psychosocial stressors examined in the current study (i.e., negative life events, daily hassles, and physical punishment) may have led to an optimal increase in arousal that in turn enhanced neurocognitive function.

Relationship between cortisol levels and neurocognitive deficits

This is the first study to investigate the relationship between cortisol levels and neurocognitive function among individuals at elevated risk for schizophrenia. The current findings indicate that the deficits in memory and executive function that have been found to characterise ASz and FHx children (Cullen et al., 2010; Dickson et al., in press-b) are associated with more abnormal HPA axis function. One possible explanation for this finding is that stress-induced HPA axis dysfunction may have a direct effect on these cognitive abilities (i.e., via the effects of cortisol on the brain structures supporting these functions). In support of this hypothesis, animal studies indicate that chronic stress exposure and persistently-elevated glucocorticoid levels cause hippocampal damage, including, dendrite atrophy, inhibition of neurogenesis, and neuronal loss (Sapolsky, 2000; Corcoran et al., 2003), and that behavioural stress leads to structural changes in the medial prefrontal cortex (Cerqueira et al., 2008). Similar associations have been observed in human populations. For example, negative correlations between cortisol levels and hippocampal volume have been observed among individuals with first-episode psychosis (Mondelli et al., 2010b), and childhood maltreatment has been associated with reduced grey matter volume in the prefrontal cortex and hippocampus in both child and adult populations (Hart & Rubia, 2012). However, the positive associations observed between experiences of psychosocial stress and neurocognitive function in the current study suggest that, if abnormal cortisol levels do directly contribute to neurocognitive deficits in ASz and FHx children, this is not driven by increased exposure or reactivity to psychosocial stressors.

Alternatively, the association between neurocognitive function and cortisol levels observed among children at elevated risk for schizophrenia may be indirect. Indeed, it is possible that the association reflects underlying dysfunction in the hippocampus

and medial prefrontal cortex, regions which are densely populated with glucocorticoid receptors and known to play a crucial role in mediating HPA axis function (Herman et al., 2005). Thus, among children at elevated risk for schizophrenia, abnormal neurodevelopmental processes (possibly triggered by genetic factors or early environmental insults) may have affected the functional integrity of the brain structures that mediate both HPA axis function and neurocognitive performance.

7.4.3 Methodological considerations

As previously, methodological issues pertinent to the data presented in this chapter are discussed here; issues that are of relevance to the study as a whole are described in the following chapter. One potential limitation relates to the use of self-report measures to assess psychosocial stress. It is possible that the positive correlations observed between neurocognitive performance and experiences of psychosocial stress may reflect the fact that children with poorer neurocognitive function were less able to recall past events and reflect on their own levels of distress. However, in the ASz group, positive correlations were observed between psychosocial stress measures and performance on executive function tasks, but not with memory performance (which would be expected if better recall was contributing to this effect). A further limitation relates to the fact that for some children, the lapse of time between the collection of psychosocial stress and salivary cortisol data and the completion of neurocognitive assessments was as long as six months. However, neurocognitive function was found to be significantly associated with both psychosocial stress and salivary cortisol measures. Moreover, the groups did not differ in the lapse of time between any of the assessments and so this would not have contributed to the different patterns of association observed across the groups.

7.4.4 Conclusions

The current study demonstrates for the first time that more abnormal HPA axis function (i.e., greater blunting of the CAR and higher diurnal cortisol levels) is associated with poorer neurocognitive function among children at elevated risk for schizophrenia. One possible interpretation of these findings is that HPA axis abnormalities, caused by increased exposure and reactivity to psychosocial stress, directly participate in the development of neurocognitive impairments in high-risk individuals. However, the positive associations observed between experiences of psychosocial stress and neurocognitive function do not support this hypothesis. Thus, it seems likely that common neurodevelopmental mechanisms may contribute to both HPA axis abnormalities and neurocognitive impairments among children at elevated risk for schizophrenia.

CHAPTER 8 Discussion

8.1 Overview

This thesis examined psychosocial stress and HPA axis function among children who are at putatively elevated risk of developing schizophrenia in later life because they present multiple antecedents of the disorder or a family history of illness. A significant methodological advance on previous studies of high-risk youth was that all children were antipsychotic-naive and were not currently seeking treatment for their symptoms. Moreover, this is the first study of individuals at elevated risk for schizophrenia to examine the CAR and also the first to investigate the extent to which experiences of psychosocial stress and cortisol levels are associated with neurocognitive function. Finally, this thesis aimed to address methodological issues identified in the existing literature by employing concurrent measures of psychosocial stress and HPA axis function and investigating the extent to which demographic factors influence the effect of risk status on these measures.

Chapter aims

This chapter provides an overview of the findings and discusses the implications of the work presented in this thesis. The specific aims were as follows:

1. Review the main findings of the study and evaluate the extent to which the results support *a priori* hypotheses.

2. Identify methodological issues that are relevant to the overall study.

3. Describe how the work presented in this thesis contributes to existing scientific knowledge.

4. Discuss the implications of the findings for theories of schizophrenia, clinical practice, and future research.

8.2 Review of the study findings

8.2.1 Principal findings

Table 37 summarises the principal findings of this thesis and indicates the extent to which the results provide support for the original study hypotheses. Of the twelve hypotheses tested, four were fully supported, five were partially supported, and three were not supported. A more detailed description of the findings is provided below.

Psychosocial stress

In Chapter 4, it was observed that FHx and ASz children are more frequently exposed to major negative life events and milder daily hassles respectively compared to TD children, and that both groups are more distressed by these experiences. These findings supported the *a priori* hypotheses and also provided evidence to suggest that ASz and FHx children may be susceptible to different types of psychosocial stressors. The results are consistent with previous studies which show that, relative to healthy controls, individuals at elevated risk for schizophrenia on account of their clinical presentation (i.e., UHR youth, individuals with SPD, and those reporting PLEs) are more likely to experience psychosocial stressors such as major life events, trauma, and daily hassles (De Loore et al., 2007; Kelleher et al., 2008; Tessner et al., 2011; Addington et al., 2013; Kelleher et al., 2013c; Sahin et al., 2013; Tikka et al., 2013). These findings also converge with studies which have observed that UHR youth (Palmier-Claus et al., 2012) and adult relatives of individuals with psychosis (Myin-Germeys et al., 2001) exhibit greater emotional reactivity to stressful experiences than healthy controls. A novel finding was that physical punishment was more common among ASz and FHx children compared to TD children. As discussed in the following section, subsequent analyses indicated that the relationship between risk status and exposure to physical punishment may be influenced by ethnicity and socioeconomic status.

The analyses conducted in Chapter 4 also indicated that exposure to psychosocial stress (negative life events, daily hassles, and physical punishment) was more strongly associated with PLEs among ASz and FHx children compared to TD children. However, larger correlations were observed between daily hassles and internalising and externalising symptoms in the TD group than in the ASz and FHx groups. Thus, whilst risk status appeared to influence the relationship between psychosocial stress and current psychopathology, the hypothesis that this association would be stronger among at-risk children was only partially supported.

Salivary cortisol

It was observed in Chapter 5 that FHx children, but not ASz children, are characterised by a blunted CAR relative to the TD group; an effect that appeared to be influenced by ethnicity (discussed in the following section). Neither FHx nor ASz children showed elevated diurnal cortisol levels relative to the TD group. Thus, only partial support was found for the hypothesis that ASz and FHx children would be characterised by abnormal cortisol levels. Whilst this is the first study to have examined the CAR among those at elevated risk for schizophrenia, a blunted CAR has also been observed in individuals with first-episode psychosis relative to healthy controls (Mondelli et al., 2010a; Pruessner et al., 2013b). The finding that neither FHx nor ASz children were characterised by elevated diurnal cortisol levels relative to their typically-developing peers is surprising given that higher cortisol levels during the day have been observed consistently among UHR youth (Sugranyes et al., 2012; Walker et al., 2013) and adolescents with SPD (Weinstein et al., 1999; Walker et al., 2001; Mittal et al., 2007). The lack of group differences may be due to the participants in the current investigation being slightly younger than those examined in previous studies of high-risk youth (i.e., it may not be possible to detect elevations in diurnal cortisol till later in adolescence).

Whilst there was partial support for the hypothesis that cortisol levels would be associated with experiences of psychosocial stress among ASz and FHx children, the pattern of findings was not as expected. The CAR was *positively* correlated with distress relating to negative life events among FHx children but *negatively* correlated in the TD group. Thus, the blunted CAR observed among FHx children was not explained by the fact that these children experienced greater distress in relation to negative life events. Interestingly, a similar pattern of findings was observed in a study of patients with first-episode psychosis (Mondelli et al., 2010a). There was no support for the hypothesis that cortisol levels would be associated with current psychopathology among ASz and FHx children. Indeed, no association was observed between cortisol levels and psychopathology in any group. Previous studies of youth at elevated risk for schizophrenia have yielded inconsistent findings; some studies have shown that cortisol levels are associated with psychotic (Weinstein et al., 1999), schizotypal (Walker et al., 2001), and prodromal symptoms (Walker et al., 2013) whilst others have not (Thompson et al., 2007; Corcoran et al., 2012; Sugranyes et al., 2012). However, the lack of association between cortisol levels and internalising and externalising psychopathology is in contrast to previous studies of youth who are not at elevated risk for psychosis (Susman, 2006; Lopez-Duran et al., 2009).

Pituitary gland volume

In contrast to hypotheses, analyses conducted in Chapter 6 indicated that there were no differences in pituitary volume when either ASz or FHx children were compared to the TD group. As noted in Chapter 2, pituitary volume abnormalities have not been consistently observed among individuals at elevated risk for schizophrenia. Enlarged pituitary volume has been reported among adults with a family history of schizophrenia (Mondelli et al., 2008) and patients with SPD (Takahashi et al., 2009). However, other studies have shown no differences between young adult relatives and

healthy controls (Habets et al., 2012), or smaller pituitary volumes among males with SPD recruited from the community (Romo-Nava et al., 2013). Furthermore, studies of UHR youth indicate that enlarged pituitary volume characterises those who go on to transition to psychosis (Garner et al., 2005; Büschlen et al., 2011), but that pituitary volumes across the total sample of UHR youth do not differ from healthy controls. Thus, differences in age, medication status, and proximity to illness onset may explain the inconsistency of findings across studies.

Pituitary volume was positively correlated with the CAR in the ASz group only, yet diurnal cortisol levels were not associated with pituitary volume in any group. Whilst these findings only partially support hypotheses, studies of individuals with a family history of psychosis (Habets et al., 2012) and youth at UHR (Thompson et al., 2007) have also reported no relationship between pituitary volume and cortisol levels during the day. There was some evidence to support the notion that pituitary volume is associated with experiences of psychosocial stress among high-risk individuals. Specifically, pituitary volume was negatively correlated with current distress relating to negative life events in the FHx group only; furthermore, FHx children who had experienced physical punishment had smaller pituitary volumes than FHx children who had not. Whilst these findings contrast with the positive association between pituitary volume and emotional stress reactivity that was observed among young adult siblings of individuals with psychosis (Habets et al., 2012), a study of adolescents with borderline personality disorder reported that those who had been exposed to childhood trauma had smaller pituitary volumes than those who had not (Garner et al., 2007). Finally, as predicted, no association was observed between pituitary volume and current psychopathology in any group. Previous studies of UHR youth (Garner et al., 2005) and patients with SPD (Takahashi et al., 2009) have also found no relationship between pituitary volume and psychotic symptoms.

Exploratory associations with neurocognitive function

Chapter 7 explored the extent to which experiences of psychosocial stress (exposure and reactivity) and cortisol levels are associated with neurocognitive function among children at elevated risk for schizophrenia. In contrast to hypotheses, greater exposure to psychosocial stress was associated with better performance on tests of memory and executive function in all three groups. Whilst some previous studies of individuals with psychosis have reported that childhood trauma is associated with greater impairments in memory and executive function (Lysaker et al., 2001; Shannon et al., 2011), other studies have observed no association between psychosocial stress exposure (including childhood maltreatment) and poorer performance in these domains (Schenkel et al., 2005; Aas et al., 2011b; Sideli et al., in press). Differences across studies may relate to the specific types of psychosocial stressor examined and/or variability in patient characteristics. Among ASz and TD children, distress relating to negative life events at the time of the event was positively associated with executive function and memory performance, respectively. Whilst this pattern of results was not predicted, the findings are consistent with two previous studies which examined the relationship between neurocognitive function and stress reactivity in individuals with psychosis using the ESM technique (Myin-Germeys et al., 2002; Morrens et al., 2007).

As hypothesised, among FHx and ASz children, more abnormal HPA axis function (that is, higher diurnal cortisol levels and a more blunted CAR) was associated with poorer performance on tests of verbal memory and executive function. This pattern of findings is consistent with studies of adults with established psychosis, in which higher diurnal cortisol levels have been associated with poorer memory and executive function (Walder et al., 2000) and a greater blunting of the CAR with poorer verbal memory (Aas et al., 2011b).

Table 37. Support for study hypotheses

	Hypothesis	Supported?	Evidence
Hypothesis 1a Chapter 4	ASz and FHx children will be exposed to higher levels of psychosocial stressors compared to the TD group	Yes	• FHx and ASz children reported a greater number of negative life events than TD children • Relative to the TD group, daily hassles more frequently experienced by ASz (peer- and teacher-related) and FHx children (home-related) • Physical punishment more common among ASz and FHx children
Hypothesis 1b Chapter 4	Relative to TD children, both ASz and FHx children will be more distressed by psychosocial stressors	Yes	• FHx children more distressed currently by negative life events than TD children • ASz children more distressed by daily hassles across all domains than TD children; FHx children more distressed by home-related hassles
Hypothesis 1c Chapter 4	Experiences of psychosocial stress will be more strongly associated with current psychopathology among ASz and FHx children than in TD children	Partially	• PLEs more strongly associated with daily hassles frequency in ASz and FHx children than in TD children; correlated with negative life events in ASz only • Daily hassles frequency and distress scores related to internalising and externalising symptoms in FHx and TD children • Physical punishment associated with internalising symptoms and PLEs among FHx children and with externalising symptoms in the TD group
Hypothesis 2a Chapter 5	ASz children and FHx children will show elevated diurnal cortisol levels and a blunted CAR relative to TD children	Partially	• FHx children showed a blunted CAR relative to TD children that was not observed among ASz children • Neither ASz nor FHx children showed elevated diurnal cortisol levels compared to TD children

Note. PLEs: Psychotic-like experiences; CAR: cortisol awakening response.

Table 37. (continued)

	Hypothesis	Supported?	Evidence
Hypothesis 2b Chapter 5	Cortisol levels will be associated with exposure to psychosocial stressors and distress related to these exposures in ASz and FHx children	Partially	• CAR positively associated with distress relating to negative life events among FHx children but negatively correlated in the TD group • Diurnal cortisol levels not associated with negative life events, daily hassles, or physical punishment in any group
Hypothesis 2c Chapter 5	Among ASz and FHx children, cortisol levels will be correlated with current psychopathology	No	• No association between cortisol levels (CAR or diurnal cortisol) and psychopathology in any group
Hypothesis 3a Chapter 6	Both ASz and FHx children will show abnormal pituitary gland volume relative to TD children	No	• Pituitary volume abnormalities not observed among ASz or FHx children relative to TD children
Hypothesis 3b Chapter 6	Among ASz and FHx children, pituitary volume will be correlated with salivary cortisol levels	Partially	• Pituitary volume positively correlated with the CAR among ASz children only • Pituitary volume not associated with diurnal cortisol levels in any group
Hypothesis 3c Chapter 6	Pituitary volume will be associated with exposure to psychosocial stressors and distress related to these exposures in ASz and FHx children	Partially	• Pituitary volume negatively correlated with current distress relating to negative life events in FHx children only • FHx children exposed to physical punishment had smaller pituitary volumes than FHx children not exposed

Note. CAR: cortisol awakening response.

Table 37. (continued)

	Hypothesis	Supported?	Evidence
Hypothesis 3d Chapter 6	No relationship between pituitary volume and current psychopathology will be observed among ASz and FHx children	Yes	• Pituitary volume not associated with any measure of psychopathology in any group
Hypothesis 4a Chapter 7	Experiences of psychosocial stress (exposure and reactivity) will be negatively associated with neurocognitive function in ASz and FHx children	No	• Experiences of psychosocial stress (exposure and reactivity) associated with better executive function among ASz children and with better memory performance among FHx and TD children
Hypothesis 4b Chapter 7	Among ASz and FHx children, more abnormal cortisol levels will be associated with poorer neurocognitive function	Yes	• Greater blunting of the CAR associated with poorer verbal memory and executive function among FHx and ASz children, but not TD children • Higher diurnal cortisol levels associated with poorer verbal memory and executive function among FHx children only

Note. CAR: cortisol awakening response.

8.2.2 Influence of demographic factors

As described in Chapter 2, a number of demographic factors have been associated with psychosocial stress exposure and HPA axis function, including, age, sex, pubertal status, ethnicity, socioeconomic status, tobacco use, and substance use. An additional aim of this thesis was to examine the role that these demographic factors play in the relationship between risk status and psychosocial stress susceptibility/HPA axis function. All primary analyses were therefore adjusted for demographic factors that differed significantly between the groups or that were associated with psychosocial stress/HPA axis function in preliminary analyses. Stratified analyses were conducted when adjustment for demographic factors led to a substantial change in the effect size associated with risk status.

Physical punishment, ethnicity, and socioeconomic status

Adjustment for demographic factors indicated that ethnicity and socioeconomic status may influence the relationship between risk status and physical punishment. Stratified analyses showed that among children of white ethnicity, physical punishment was more commonly experienced by ASz and FHx children compared to the TD group, yet there was no effect of ASz and FHx status on physical punishment among those of non-white ethnicity. A similar pattern was observed when analyses were conducted within socioeconomic strata; among children of higher socioeconomic class, but not those of lower social class, ASz and FHx children were more likely than TD children to experience physical punishment. These findings may reflect the fact that physical punishment was more commonly experienced by children of non-white ethnicity and those from low socioeconomic backgrounds. Thus, the extent to which physical punishment is associated with risk for schizophrenia in children from different ethnic and socioeconomic backgrounds may depend on how prevalent these experiences are in particular demographic groups.

Cortisol awakening response, gender, and ethnicity

There was also evidence that ethnicity may influence the relationship between risk status and the cortisol awakening response. Specifically, among children of non-white ethnicity, FHx children were characterised by a blunted CAR relative to the TD group, but there was no difference in the CAR when white FHx children were compared to white TD children. Stratified analyses showed that the blunted CAR observed among FHx children relative to the TD group was present in both males and females, thus, the effect of FHx status on the CAR was not influenced by gender. It is possible that the modifying effect of ethnicity may have reflected the fact that the proportion of FHx children with a first-degree relative with schizophrenia was higher among those who were of non-white ethnicity compared to those of white ethnicity. In Chapter 5, it was also demonstrated that the blunted CAR observed among FHx children relative to the TD group was more pronounced among FHx children with a first-degree relative with schizophrenia compared to those with an affected second-degree relative. Importantly, the stratified analyses demonstrate that the blunted CAR observed among FHx children is not simply due to the fact that these children are more likely to be of non-white ethnicity.

Pituitary volume, sex, and pubertal status

Stratified analyses were also conducted to examine the influence of sex and puberty on the relationship between risk status and pituitary volume. These analyses demonstrated that neither ASz nor FHx children differed to the TD group on pituitary volume when males and females were examined separately, or when analyses were conducted within pubertal strata (pubertal vs. post-pubertal). Thus, there was no evidence to suggest that sex or pubertal status modified the relationship between risk status and pituitary volume in this sample.

8.3 Methodological considerations

Methodological issues relating to the specific analyses conducted in Chapters 4, 5, 6, and 7 have been discussed previously. The following section provides a more comprehensive description of the issues that are relevant to the overall study.

8.3.1 Sample constraints

The current study was limited by the small sample size, which may have impaired the ability to detect significant group differences. This was a particular concern for the pituitary volume analyses, as the sample size was further reduced owing to the fact that some children did not wish to complete an MRI scan or were unable to do so due to contraindicators (e.g., metal braces). Small sample sizes are not uncommon in high-risk studies, particularly those examining biological indices of HPA axis function. This reflects the inherent difficulties in identifying and recruiting high-risk youth, as well as the relative intrusiveness of obtaining biological measures compared to self-report questionnaire data. Despite the small sample, the current study was able to detect differences between the at-risk groups and the TD group on measures of psychosocial stress (exposure and reactivity) and the CAR. Furthermore, within the FHx group, significant differences in pituitary volume were observed between those who had experienced physical punishment and those who had not.

A further limitation relates to the extent to which the groups examined in the current study are representative of the underlying populations from which they were sampled. Potential ASz and TD cases who declined participation did not differ on age, sex, ethnicity, or psychopathology at screening to ASz and TD children who participated in the overall study respectively, with the exception that ASz participants were less likely to present SDQ emotional symptoms in the 'abnormal' range than children meeting ASz criteria who did not participate. Given that depression has been associated with HPA axis abnormalities, it is possible that the

under-representation of ASz children reporting emotional problems may have influenced the results of the study. A related concern is whether the study findings can be extended to other populations. Within the inner-city London boroughs from which the participants were sampled, environmental risk factors for psychosis (for example, urbanicity and migration) are likely to be particularly prevalent. Indeed, the incidence of psychosis in south-east London (where many of the participants reside) is higher than the incidence in other parts of the UK (Kirkbride et al., 2012). It is possible that these environmental risk factors may have acted in combination with ASz and FHx status, thereby contributing to the observed associations between risk status and psychosocial stress susceptibility/HPA axis function.

The fact that the groups were not matched on key demographic variables might also be considered a potential limitation of the study. Compared to the TD group, ASz and FHx children were more likely to be of black or other ethnicity and tended to be from lower socioeconomic backgrounds. This pattern is consistent with the elevated rates of psychosis observed among African-Caribbean and black African adults in the UK (Kirkbride et al., 2012), and with population-based studies showing that low socioeconomic status in childhood is associated with increased risk for psychosis (Wicks et al., 2005; Corcoran et al., 2009). ASz children were also more likely to be male than TD children, which again, is in accordance with the finding that males are at greater risk of developing schizophrenia (Tandon et al., 2009). Thus, the demographic factors that distinguished ASz and FHx children from the TD group are also factors on which individuals with schizophrenia and healthy controls differ. It is also important to note that the TD group do not represent a 'super-healthy' control group. By definition, TD children could present scores in the 'borderline' range on the SDQ psychopathology scales (i.e., scores within the 80-90th percentile on UK population norms) and could report multiple 'somewhat-true' PLEs.

8.3.2 Measurement issues

The current study examined a range of child-appropriate negative life events and school-related daily hassles. A potential limitation is that more severe forms of psychosocial stress such as childhood trauma (i.e., abuse and neglect) were not examined. Childhood trauma is associated with increased risk for schizophrenia (Varese et al., 2012; Matheson et al., 2013a) and has also been related to HPA axis abnormalities in adolescents, as indexed by a blunted cortisol response to psychosocial stress (Ouellet-Morin et al., 2011) and reduced pituitary volume (Garner et al., 2007). Thus, childhood trauma might have explained the blunted CAR observed among FHx children. However, the ability to examine physical punishment, which lies on a continuum with childhood maltreatment, is a potential strength of the study. The findings indicate that even at lower levels of severity, physically abusive parenting is associated with pituitary volume reduction among vulnerable youth.

Salivary cortisol was used to index HPA axis function. Given that cortisol levels show substantial variation from day-to-day, the measurement of cortisol in hair samples may have provided a more stable indication of HPA axis hyperactivity (Russell et al., 2012). However, the reliability and validity of hair cortisol measures are yet to be determined, and the current study observed that salivary cortisol values were stable across the two testing days. Additionally, the saliva sampling protocol permitted the examination of the CAR and diurnal cortisol variation, neither of which can be captured using hair samples. An alternative to using cortisol to assess HPA axis function is to examine adrenocorticotropic hormone (ACTH) levels. Indeed, elevated ACTH levels have also been observed among individuals with schizophrenia relative to healthy controls (Walker et al., 2008). However, ACTH is typically examined in blood plasma, and the invasiveness of venipuncture limits the ability to collect plasma samples in child populations.

Pituitary gland volume was used as an additional proxy measure of HPA axis activity. However, pituitary volume was unrelated to cortisol levels in the current study, which is consistent with previous studies of high-risk individuals (Thompson et al., 2007; Habets et al., 2012). Whilst an alternative would have been to examine hypothalamic volume, the few studies that have compared hypothalamic volume in individuals with schizophrenia and healthy controls have yielded inconsistent findings (Goldstein et al., 2007; Koolschijn et al., 2008; Klomp et al., 2012), and no study has yet examined the relationship between hypothalamic volume and cortisol levels. It has also been suggested that hippocampal volume might be used to index HPA axis activity (Walker et al., 2008; Aiello et al., 2012). Indeed, studies of individuals with first-episode psychosis have reported an inverse relationship between cortisol levels and hippocampal volume (Mondelli et al., 2010b). However, other factors, including pregnancy and birth complications, cannabis use, and genetic variation may also contribute to reduced hippocampal volume (Stefanis et al., 1999; Hass et al., 2013; James et al., 2013).

8.3.3 Analytical issues

Multiple testing is a major limitation of the current study. Given the small group sizes, a reasonably liberal statistical threshold was employed for all analyses ($p \leq 0.05$, uncorrected); thus, statistically significant results may have arisen by chance. In total, 30 regression analyses examining group differences (ASz vs. TD and FHx vs. TD) in psychosocial stress and HPA axis function were conducted. Employing this statistical threshold, two significant results might be expected by chance; in fact, significant differences were observed in 12 analyses. However, a large number of correlation analyses were conducted with no corrections applied to reduce the risk of type 1 errors, so some of the significant correlations observed may have been spurious. These investigations are exploratory in nature, and must be interpreted with caution.

The cross-sectional analyses employed in the current study limit the extent to which causal inferences can be made regarding the observed associations. Whilst the effect of risk status on psychosocial stress susceptibility and HPA axis function was examined prospectively (i.e., these factors were assessed approximately two years after initial identification of at-risk children), for the ASz group in particular, these factors may have contributed to schizophrenia vulnerability. As such, it is not clear whether exposure to stressful events in early life led to the psychopathology which characterised ASz children at identification (i.e., internalising and externalising symptoms and PLEs), or whether these symptoms increased the likelihood that ASz children would encounter psychosocial stressors. The cross-sectional nature of these analyses means that it is not possible to disentangle the temporal relationship between risk status and psychosocial stress exposure. Although as noted previously, these associations are likely to be bidirectional.

A final limitation of the current study relates to the fact that the ASz and FHx groups were not mutually exclusive and so could not be directly compared to each other. The decision was taken to include children meeting ASz and FHx criteria in both groups in order to most accurately reflect the ASz and FHx populations from which these participants were sampled. Owing to the small sample size, there were insufficient numbers to examine children meeting both ASz and FHx criteria as a separate group. It is possible that children who present both developmental and genetic risk factors may be at even greater risk of developing schizophrenia than children who present either feature in isolation, therefore ASz+FHx children might show even more pronounced differences in psychosocial stress susceptibility and HPA axis function relative to the TD group. Regrettably, it was not possible to test this hypothesis in the current study.

8.4 Scientific contribution

8.4.1 Characterising individuals at elevated risk for schizophrenia

The current study examined a range of psychosocial stressors and biological markers of HPA axis function, some of which had not been previously assessed in a high-risk sample. The work presented in this thesis therefore provides new information on the characteristics of children at elevated risk for schizophrenia and identifies features that these children share with adults with established illness.

Children with a family history of schizophrenia

Individuals with a family history of schizophrenia have been intensively studied over the past fifty years; however, few studies have examined experiences of psychosocial stress in this population. The current study demonstrates that FHx children are characterised by increased exposure to psychosocial stressors (major negative life events and physical punishment) relative to their typically-developing peers, and that they are more distressed by these experiences. In contrast, two previous studies, one of youth with a family history of schizophrenia (Miller et al., 2001), and the other of adult siblings (Myin-Germeys et al., 2001), found no evidence that relatives were more likely than healthy controls to experience major life events or minor stressors throughout the day. However, the latter study reported that relatives showed greater emotional reactivity to stressful events, which is consistent with the current findings.

The current study is the first to examine the CAR among individuals with a family history of schizophrenia. The finding that FHx children were characterised by a blunted CAR relative to the TD group is consistent with previous studies showing that individuals with a family history of illness share a number of neurobiological characteristics with their affected relatives, including, neuromotor abnormalities (Niemi et al., 2003), cognitive deficits (Sitskoorn et al., 2004; Agnew-Blais & Seidman,

2013), and structural brain abnormalities (Fusar-Poli et al., in press). In contrast, FHx children did not show elevations in diurnal cortisol levels or pituitary volume abnormalities compared to the TD group. Whilst some previous studies have observed these features among adult relatives of individuals with psychosis (Mondelli et al., 2008; Collip et al., 2011; Yıldırım et al., 2011) others have not (Marcelis et al., 2004; Spelman et al., 2007; Brunelin et al., 2008; Yıldırım et al., 2011; Habets et al., 2012; Yang et al., 2012). The reason for the inconsistency across studies is unclear, although it is possible that demographic factors and the presence of 'schizotypal' psychopathology among relatives may play a role.

This study also demonstrated for the first time that the neurocognitive impairments observed among youth with a family history of schizophrenia (Agnew-Blais & Seidman, 2013) may be associated with more abnormal HPA axis function. Among FHx children, a more blunted CAR was associated with poorer verbal memory and higher diurnal cortisol was negatively correlated with performance on measures of memory and executive function. These findings are consistent with those of previous studies that have observed a relationship between neurocognitive performance and HPA axis function among adults with psychosis (Walder et al., 2000; Aas et al., 2011b).

Children presenting multiple antecedents of schizophrenia

The current study also examined children at putatively elevated risk for schizophrenia who present multiple antecedents of the disorder. These children were identified using a novel community-based screening method that was designed to enable the identification of high-risk youth at an earlier stage of illness than the UHR approach (Laurens et al., 2007; Laurens et al., 2011). It was additionally hoped that this identification strategy would address the generalisability issues associated with studies of individuals with a family history of illness. The current study demonstrates

that, like individuals with schizophrenia, ASz children are more likely to experience psychosocial stressors than their typically-developing peers (daily hassles, negative life events, and physical punishment) and show greater reactivity to these experiences. These findings are consistent with previous studies of youth at 'symptomatic-risk' for schizophrenia (De Loore et al., 2007; Kelleher et al., 2008; Tessner et al., 2011; Palmier-Claus et al., 2012; Phillips et al., 2012; Addington et al., 2013; Kelleher et al., 2013c; Sahin et al., 2013; Tikka et al., 2013).

In contrast, ASz children were not characterised by abnormal HPA axis function (either a blunted CAR, elevated diurnal cortisol levels, or pituitary volume abnormalities) relative to TD children; although greater blunting of the CAR was associated with poorer verbal fluency in this group. These findings contrast with previous investigations by our group which show that ASz children present several neurobiological features that characterise adults with schizophrenia, including, functional brain abnormality following commission of behavioural errors (Laurens et al., 2010), neurocognitive impairments (Cullen et al., 2010), involuntary dyskinetic movement abnormalities (Macmanus et al., 2012), and structural brain abnormalities encompassing the temporal lobe (Cullen et al., 2013), relative to TD children. It is possible that these features represent markers of abnormal neurodevelopment caused by genetic and early environmental risk factors and that HPA axis abnormalities may not emerge till later in the disease process.

Potential mechanisms

It is likely that a range of co-occurring environmental factors, including, social disadvantage and ethnic minority status, contribute to the higher levels of psychosocial stress experienced by ASz and FHx children. For those FHx children who have an unwell parent, additional factors, such as parental substance abuse and social isolation (Campbell et al., 2012), may facilitate some of these experiences. As

such, these children may be living in environments which have contributed to both their elevated risk state and to the probability of stressful events occurring. However, ASz and FHx status may play a more active role in increasing the risk for psychosocial stress exposure. For example, the higher levels of psychopathology presented by ASz children may influence the way in which they interact with their peers, teachers, and family members, and increase the likelihood that they will experience daily hassles in their lives. It is also possible that the characteristics of ASz and FHx children in some way contributes to the higher levels of physical punishment that they experience. Thus, schizophrenia risk status may lead to greater exposure to psychosocial stressors via both indirect and direct pathways.

The finding that FHx children exhibited greater levels of distress currently in relation to negative life events than TD children, but that their ratings of distress at the time of the event were not significantly higher, may imply that FHx children used less adaptive coping mechanisms following exposure to these events, causing their distress to persist at higher levels over time. For example, lower levels of social support (Ozer et al., 2003) and impairments in attention and executive functioning (Aupperle et al., 2012) have been associated with the subsequent development of PTSD symptoms among people exposed to trauma. It is possible that these factors may contribute to poorer coping abilities among FHx children. The heightened sensitivity to daily hassles that characterised ASz children relative to the TD group may be caused by different factors. One possible explanation is that dopamine dysregulation, which is thought to underlie hallucinatory and delusional experiences by contributing to aberrant attribution of salience to environmental stimuli (Kapur, 2003), may cause these minor events to be more salient to ASz children. Thus, both neurobiological and cognitive mechanisms may contribute to the increased reactivity to psychosocial stressors that was found to characterise both FHx and ASz children.

The blunted CAR observed among FHx children, but not ASz children, relative to the TD group may reflect a genetically-mediated effect. Several lines of evidence lend support to this hypothesis. Firstly, the current study observed that the effect of FHx status was twice as large among those with a first-degree relative with schizophrenia compared to those with an affected second-degree relative. Secondly, the blunted CAR observed among FHx children was not explained by any of the environmental stressors examined in the current study. Thirdly, there is evidence that the CAR (Wust et al., 2000) and awakening and post-awakening cortisol levels (Bartels et al., 2003) are influenced by genetic factors. Moreover, a single-nucleotide polymorphism that plays a role in the aetiology of depression (rs2522833 within the *PCLO* gene) has also been associated with a blunted CAR (Kuehner et al., 2011). However, the presence of a blunted CAR among FHx children does not necessarily imply that this is a genetically-mediated effect; this characteristic might equally be driven by other environmental exposures that were not examined in the current study.

One possible explanation for the finding that more abnormal cortisol levels were associated with poorer memory and executive function among FHx and ASz children is that stress-induced HPA axis dysfunction has a direct effect on the brain structures supporting these functions. However, the positive correlations observed between psychosocial stress exposure and neurocognitive function (and indeed, the lack of association between cortisol levels and psychosocial stress) suggests that the relationship between neurocognitive function and cortisol is not driven by an excess of psychosocial stressors. Instead, it seems likely that the relationship between neurocognitive function and cortisol is indirect. It is possible that abnormal neurodevelopmental processes (commencing in early life) may have affected the functional integrity of the brain structures mediating both HPA axis function and neurocognitive performance (i.e., the hippocampus and medial prefrontal cortex).

8.4.2 Aetiology of schizophrenia

One of the primary motivations for studying individuals at elevated risk for schizophrenia is that it offers the opportunity to identify disease characteristics that precede illness onset and contribute to the development of psychosis. The current study provides further insights into the nature and timing of psychosocial stress susceptibility and HPA axis abnormalities in schizophrenia.

Early markers of psychosis vulnerability

Studies examining youth at UHR, individuals with SPD, and adult relatives of individuals with schizophrenia indicate that these populations are characterised by increased exposure and reactivity to psychosocial stress and HPA axis dysfunction. Whilst these studies provide some evidence to suggest that psychosocial stress and HPA axis abnormalities precede psychosis onset, characteristics of the high-risk samples examined to date limit the ability to draw this conclusion. The current study aimed to address these limitations by examining high-risk children who are antipsychotic-naive and not currently seeking help for their symptoms. The current findings indicate that some of the psychosocial stress and HPA axis abnormalities that characterise older samples of high-risk youth are present during the early stages of illness, whilst others are not. Specifically, there is evidence that high-risk children are more likely to experience psychosocial stress than healthy children and show increased reactivity to these events, and that FHx children are characterised by a blunted CAR. However, neither ASz nor FHx children showed elevated diurnal cortisol levels or pituitary volume abnormalities. These findings imply that increased susceptibility to psychosocial stress (i.e., increased exposure and greater reactivity) and a blunted CAR may constitute early markers of psychosis vulnerability, whilst other indices of HPA axis dysfunction (i.e., elevated diurnal cortisol levels and abnormal pituitary volume) may not emerge till closer to disease onset.

Potential mechanisms leading to disease onset

According to cognitive models, psychosocial stress may lead to psychosis via emotional and cognitive pathways (Garety et al., 2007). It is proposed that among vulnerable individuals, stress can trigger emotion dysregulation (heightened anxiety and depression) and cognitive dysfunctions (e.g., negative beliefs about the self and others) which lead to anomalous experiences; psychosis results when these anomalous experiences are attributed to external causes. In support of the model, a study of young adults found that the association between childhood trauma and PLEs was partially mediated by negative beliefs about the self and others (Gracie et al., 2007). Furthermore, a large prospective study observed that the relationship between psychosocial stress exposure in childhood (harsh parenting, bullying, and domestic violence) and PLEs in adolescence was mediated by anxiety, depressive symptoms, external locus of control, and low self-esteem (Fisher et al., 2013b). Exposure to childhood trauma may also contribute to the deficits in social functioning that characterise individuals with schizophrenia (Stain et al., in press); it is possible that traumatic experiences impair the ability to develop key social skills necessary for developing and maintaining relationships.

Of course another mechanism by which psychosocial stress may contribute to the onset of psychosis is via the HPA axis. It has been proposed that stress-induced HPA axis dysfunction leads to dopamine dysregulation which in turn gives rise to psychosis (Walker et al., 2008). Although abnormal HPA axis function has been observed among individuals with schizophrenia and those at elevated risk for the disorder, it is not clear whether these abnormalities are triggered by psychosocial stress exposure. However, in support of this model, a study using the ESM technique observed that the effect of stressful events on psychotic symptoms among adult relatives of individuals with psychosis was modified by homovanillic acid reactivity

(indexing dopamine responsivity), yet this relationship was not observed among healthy controls (Myin-Germeys et al., 2005b). These findings suggest that, among individuals at elevated risk for schizophrenia, abnormal dopamine reactivity may contribute to psychotic symptoms following exposure to psychosocial stress.

Finally, it has been acknowledged that psychosocial stress may contribute to psychosis indirectly by increasing substance use behaviours, which in turn confer risk for psychosis (Fisher, 2013). Indeed, a large population-based study of adults reported that the association between childhood trauma and hallucinations was partially mediated by substance use (Whitfield et al., 2005). However, a significant relationship between childhood trauma and hallucinations was observed among individuals with substance use histories and those without, suggesting that substance use is not the only mechanism by which psychosocial stress leads to psychosis.

Very little is known about the functional significance of the blunted CAR. One suggestion is that the CAR reflects anticipation of the demands of the upcoming day (Fries et al., 2009), although it is not clear whether the CAR directly influences the ability to deal with stressors. Alternatively, the CAR can be interpreted as the cortisol response to a minor stressor (i.e., awakening). As such, the blunted CAR observed among individuals with first-episode psychosis (Mondelli et al., 2010a; Pruessner et al., 2013b), and in the current study, among FHx children, may be similar to the blunted cortisol responses to psychosocial stressors reported in individuals with schizophrenia (Jansen et al., 2000; Brenner et al., 2009) and youth at UHR (Pruessner et al., 2013a). It has been suggested that among those who are at elevated risk for psychosis, attenuated cortisol responses to stress might aggravate underlying vulnerability and eventually lead to psychosis (Pruessner et al., 2013a). However, a comprehensive explanation for how a blunted CAR might contribute to the development of schizophrenia is currently lacking.

Specificity for schizophrenia outcome

This thesis has focused on the extent to which psychosocial stress and HPA axis abnormalities are early vulnerability markers for schizophrenia and psychotic disorders. However, it is possible that these markers may also confer vulnerability for other psychiatric disorders. FHx and ASz children may develop other psychiatric disorders in later life, not just schizophrenia. For example, a large population-based study observed that offspring of individuals with psychosis are at greater risk for a number of adult outcomes, including, psychosis, bipolar disorder, depression, anxiety disorders, personality disorders, and substance misuse disorders (Dean et al., 2010). Thus, the risk conferred by having a family history of psychosis is not limited to concordant disorders. Whilst the diagnostic outcomes for ASz children are not yet known, it seems likely that these children are also at risk for a number of mental health problems. Longitudinal studies show that PLEs predict non-psychotic outcomes, including, major depression, anxiety disorders, and mania (Kaymaz et al., 2012), although the association with psychotic disorder is much stronger. Similarly, internalising and externalising problems in childhood are associated with a range of adult mental problems (Cannon et al., 2002; Kim-Cohen et al., 2003). Speech and motor problems in infancy, however, appear to be more specifically related to psychosis (Isohanni et al., 2001; Cannon et al., 2002). Thus, the individual antecedents that comprise the triad confer risk for a number of mental health problems, although using a combination of antecedents may provide a more specific and sensitive means of identifying children who will later develop schizophrenia.

Psychosocial stress susceptibility and HPA axis abnormalities have likewise been implicated in other psychiatric disorders. Studies have consistently observed that childhood trauma and major life events are associated with increased risk for major depression (Young & Korszun, 2010; Nanni et al., 2012; Lindert et al., in press), and

studies employing the ESM technique show that individuals with depression are characterised by increased emotional reactivity to daily stressors relative to healthy controls (aan het Rot et al., 2012). Furthermore, studies conducted over the past four decades indicate that individuals with depression are characterised by elevated diurnal cortisol levels (Stetler & Miller, 2011), although there is some evidence to suggest that the depression sub-types (melancholic vs. atypical) are associated with different patterns of HPA axis function, with increased cortisol being a feature of melancholic depression specifically (O'Keane et al., 2012). Whilst the CAR has been less frequently examined in this population, several studies have observed an increased CAR among both adolescents and adults with depression relative to healthy controls (Bhagwagar et al., 2003; Dienes et al., 2013; Ulrike et al., 2013). Thus, the combination of HPA axis abnormalities in major depression (elevated diurnal cortisol levels and an increased CAR) is different to that observed among individuals with psychosis (elevated diurnal cortisol levels and a blunted CAR). Another disorder that has been associated with HPA axis abnormalities is PTSD, which, by definition, involves exposure to trauma. There is evidence that individuals with PTSD, like those with psychosis, are characterised by a blunted CAR (Chida & Steptoe, 2009). However, studies have also consistently shown that PTSD is associated with decreased cortisol levels during the day relative to healthy controls (Morris et al., 2012). Crucially, although psychosocial stress and HPA axis abnormalities have been implicated in other psychiatric disorders, the pattern of HPA axis abnormalities is not the same as that observed among individuals with psychosis. Thus, the blunted CAR that was found to characterise FHx children (in the absence of decreased diurnal cortisol levels, as would be consistent with PTSD) may constitute a relatively specific marker of psychosis risk. It is possible that genetic influences contribute to the distinct pattern of HPA axis dysfunction observed among individuals with psychosis.

8.5 Implications

8.5.1 Implications for existing theories

Neural diathesis-stress model of schizophrenia

The neural diathesis-stress model has emerged as one of the leading aetiological theories of schizophrenia (Walker & Diforio, 1997; Walker et al., 2008). The model proposes that individuals with an underlying vulnerability for the disorder are more susceptible to experiences of psychosocial stress, and that the HPA axis (via the augmenting effects of cortisol on dopamine activity) mediates the relationship between these experiences and the development of psychosis. Studies examining individuals with schizophrenia and those at elevated risk for the disorder have provided some evidence to support the model, yet few have employed multiple measures of psychosocial stress and HPA axis function. By addressing this limitation, the current study was able to directly test several key components of the model.

The current study provides support for the notion that children at elevated risk for schizophrenia are more susceptible to psychosocial stress than children who are at low risk for the disorder. However, there was no evidence to suggest that these experiences led to increased HPA axis activity (i.e., elevated diurnal cortisol levels and enlarged pituitary volume), as would be proposed by the model. Indeed, the blunted CAR observed among FHx children was not explained by any of the psychosocial stressors examined, and the pattern of association between psychosocial stress and HPA axis function was not as expected (i.e., among FHx children, the CAR was positively correlated with distress relating to negative life events whilst pituitary volume was negatively associated with distress relating to negative life events and exposure to physical punishment). However, it is important to stress that the current findings do not preclude the possibility that stress-induced HPA axis hyperactivity contributes to the onset of psychosis; as none of the children

in the current study have yet experienced a psychotic episode, this could not be examined. Given that the brain undergoes significant structural changes during adolescence and that HPA axis activity also increases during this time, it is possible that psychosocial stressors experienced in later adolescence have a greater impact on HPA axis function, and that these experiences are thus able to trigger psychosis onset.

Clinical staging model of psychosis

The current findings are also relevant to the clinical staging model of schizophrenia (McGorry et al., 2006; Yung & McGorry, 2007; Wood et al., 2011), which proposes that the development of psychosis can be conceptualised as a series of stages that correspond to the extent, progression, and impact of the illness. A key feature of the model is that advancement through the clinical stages should correspond to progressive neurobiological changes, such that the later stages of illness are characterised by more pronounced neurobiological abnormalities.

ASz children would be classed within Stage 1a of the model (Table 2, Chapter 1), as would some FHx children (i.e., those presenting some or all of the developmental antecedents), although children with a family history of illness without accompanying psychopathology would currently be classified as Stage 0. Given that ASz and FHx children were not characterised by elevated diurnal cortisol levels and pituitary volume abnormalities, but that these features have been observed among some UHR youth and individuals with SPD, it is tempting to speculate that HPA axis hyperactivity does not emerge until at least Stage 1b of illness (which includes UHR youth and thus some individuals with SPD). In contrast, the blunted CAR observed among FHx children may be a genetically-mediated liability marker that is not necessarily associated with disease progression. Although tentative, the current study lends support to the idea that more advanced stages of psychosis are associated with more abnormal HPA axis function.

8.5.2 Clinical implications

The finding that ASz and FHx children showed increased exposure and reactivity to psychosocial stress, but were not yet characterised by HPA axis hyperactivity, indicates that this may be a crucial time to implement early intervention strategies. Broadly speaking, there are two ways to approach this. One strategy is to focus on interventions aimed at helping vulnerable youth to deal more effectively with psychosocial stress, which may help to reduce the impact of these psychosocial stressors on HPA axis function. Alternatively, preventative interventions may help to reduce the risk of exposure to psychosocial stressors among these youth.

Psychological therapies to promote coping strategies

A number of psychological therapies have been found to help children cope with major traumatic events. For example, child-parent psychotherapy has been shown to reduce PTSD symptoms and internalising and externalising problems among children exposed to trauma (Ghosh Ippen et al., 2011), and there is evidence that cognitive behavioural therapies are effective in reducing psychopathology among children who have experienced sexual abuse (Ramchandani & Jones, 2003). The current study indicates that the death of a loved one is a particularly common experience in childhood. Whilst the effect sizes are relatively small, bereavement interventions for children have been associated with improvements in coping behaviours, with music therapy emerging as a promising treatment model (Rosner et al., 2010).

School-based stress education programmes

An intervention strategy which is particularly relevant to the current study is a novel school-based education programme *(DeStress for Success)* designed to reduce the risk of depression in adolescents (Lupien et al., 2013). The programme has been informed by recent research into the relationship between HPA axis function and depression, and aims to enable adolescents to deal more effectively with psychosocial stress. The

intervention was recently trialled in a sample of 504 high school students, and was found to lead to a reduction in cortisol levels among adolescents who exhibited high levels of anger at baseline (Lupien et al., 2013). Moreover, adolescents who showed a decrease in cortisol levels over the treatment period were at lower risk of experiencing depression at a three-month follow-up than adolescents who did not. These promising findings suggest that psychosocial interventions might be used to target HPA axis abnormalities in individuals at elevated risk for psychosis.

Preventative programmes

An alternative strategy is to employ preventative interventions to reduce the risk of exposure to psychosocial stressors. For example, studies in the United States have consistently shown that home-visitation programmes targeting vulnerable families (i.e., low-income families, first-time mothers, and families experiencing stress and difficulties) can successfully prevent childhood maltreatment (Macmillan et al., 2009). In addition, a number of school-based prevention programmes have been found to reduce levels of bullying in schools (Rigby & Slee, 2008). Thus, there is evidence that intervention programmes can successfully prevent some of the psychosocial stressors that are most strongly associated with psychosis vulnerability.

8.5.3 Directions for future research

Further investigations in ASz and FHx children

The work presented in this thesis has generated several hypotheses that can be tested within this cohort of at-risk children. An important finding was that more abnormal HPA axis function is associated with poorer performance on memory and executive function tasks among ASz and FHx children. It is assumed that this relationship is largely mediated by the hippocampus and the medial prefrontal cortex (which play a major role in regulating HPA axis function), although this has not yet been directly tested. Work has already commenced on the study to obtain

hippocampal volumes from structural brain images using semi-automated extraction techniques. The current findings indicate that it will be important to examine this data in conjunction with measures of HPA axis function and neurocognitive function (particularly memory) in order to determine whether hippocampal volume mediates the relationship between these measures.

None of the psychosocial stress measures examined in the current study were able to explain the blunted CAR observed among FHx children relative to the TD group. For ethical reasons, participants were not asked about experiences of maltreatment whilst aged less than 16 years. However, we have recently commenced a fourth assessment phase (age 17-18 years) where we will collect information on a range of victimisation experiences which will allow us to retrospectively ascertain exposure to abuse and neglect in early life. Thus, it will be possible to determine whether more severe forms of psychosocial stress can explain the blunted CAR observed among FHx children.

The findings of the current study suggest that HPA axis hyperactivity (as indexed by elevated diurnal cortisol levels and enlarged pituitary volume) is a feature that may emerge closer to psychosis onset. At the fourth assessment phase, we will assess UHR status using the CAARMS (Yung et al., 2005); an important question arising from the current study is whether HPA axis hyperactivity is present among FHx and ASz children who meet UHR criteria at follow-up. For example, longitudinal studies of UHR youth have shown that those who later transitioned to psychosis (but not those who did not) showed enlarged pituitary volume at baseline (Garner et al., 2005; Büschlen et al., 2011). The current findings also emphasise the importance of examining cortisol levels at the next assessment phase in order to determine whether HPA axis hyperactivity increases over time in those who progress to more advanced stages of illness.

Epidemiological studies

The current investigation has identified a number of associations which require replication in a larger epidemiological study. Studies conducted in small, enriched samples of high-risk individuals are limited in the extent to which they can examine causal mechanisms. Such studies also impose categorical constructs (i.e., high-risk vs. low-risk individuals), when in reality, liability for schizophrenia is distributed throughout population. A large 'unselected' population cohort would provide the opportunity to more definitively determine whether antecedents of schizophrenia increase the risk for experiences of psychosocial stress and HPA axis dysfunction. For example, a continuous measure of liability (i.e., the number of antecedents) could be used to determine whether there is a dose-response relationship between the triad components and these outcomes. In a larger sample, it would also be possible to investigate whether those presenting antecedents *and* a family history of illness are at even greater risk for psychosocial stress/HPA axis abnormalities.

Sample constraints also limited the ability to examine the role that demographic factors may play in the relationship between risk status and psychosocial stress/HPA axis function. One novel finding of the current study was that ethnicity and socioeconomic status appear to influence the effect of risk status on physical punishment and the CAR. Although the current sample was not sufficiently large to be able to adequately test for effect modification, these exploratory analyses suggest that the role of demographic factors should be investigated further.

Despite the limitations, it is important to stress that studies conducted in small, enriched high-risk samples provide the opportunity to conduct assessments that cannot be easily employed in large samples (e.g., MRI scans). The current study has generated a number of intriguing findings that can inform future investigations in larger population-based cohorts.

8.6 Final conclusions

The work presented in this thesis suggests that increased susceptibility to psychosocial stress and a blunted cortisol awakening response may constitute early markers of psychosis vulnerability. By examining children with different vulnerability profiles for schizophrenia, the current study also provided evidence that might be interpreted to suggest that the blunted CAR may be a genetically-mediated marker of vulnerability. The current study also demonstrated that the effect of risk status on psychosocial stress susceptibility and HPA axis function may be influenced by ethnicity and socioeconomic status. These preliminary findings require replication in larger 'unselected' population cohorts. Given that the current study found no evidence that children at elevated risk for schizophrenia were characterised by HPA axis hyperactivity, it is speculated that this feature may not emerge until more advanced stages of illness. Interventions aimed at helping at-risk youth to cope more effectively with psychosocial stress might prevent the development of further HPA axis abnormalities and avert transition to psychosis.

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APPENDICES

Appendix 1. Child screening questionnaire

Code: «School» - «Pupil» - «Class»

Questionnaire for children aged 9-12 years

Please follow along as the questions are read out to you. Each time we read an item, please think carefully about it before you answer. Please mark your answer in the box like this: ☑ Let's start with some easy ones to help you practice:

A. How old are you?	**9 years or younger** ☐	**10 years** ☐	**11 years** ☐	**12 years or older** ☐
B. Which are you?	**a Boy** ☐	**a Girl** ☐		

From now on, please mark the box for Not True, Somewhat True, or Certainly True. It would help us if you answered all the items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of how things have been for you *over the last six months*.

	Not True	Somewhat True	Certainly True
Example: I always wear a watch	☐	☐	☐
1. I try to be nice to other people. I care about their feelings	☐	☐	☐
2. I am restless. I cannot stay still for long	☐	☐	☐
3. I get a lot of headaches, stomach-aches, or sickness	☐	☐	☐
4. I usually share with others (food, games, pens, etc.)	☐	☐	☐
5. I get very angry and often lose my temper	☐	☐	☐
6. I am usually on my own. I generally play alone or keep to myself	☐	☐	☐
7. I usually do as I am told	☐	☐	☐
8. I worry a lot	☐	☐	☐
9. I am helpful if someone is hurt, upset, or feeling ill	☐	☐	☐
10. I am constantly fidgeting or squirming	☐	☐	☐
11. I have one good friend or more	☐	☐	☐
12. I fight a lot. I can make other people do what I want	☐	☐	☐
13. I am often unhappy, down-hearted, or tearful	☐	☐	☐
14. Other people my age generally like me	☐	☐	☐
15. I am easily distracted. I find it difficult to concentrate	☐	☐	☐
16. I am nervous in new situations. I easily lose confidence	☐	☐	☐
17. I am kind to younger children	☐	☐	☐
18. I am often accused of lying or cheating	☐	☐	☐
19. Often children or young people pick on me or bully me	☐	☐	☐
20. I often volunteer to help others (parents, teachers, children)	☐	☐	☐
21. I think before I do things	☐	☐	☐
22. I take things that are not mine from home, school, or elsewhere	☐	☐	☐
23. I get on better with adults than with people my own age	☐	☐	☐
24. I have many fears. I am easily scared	☐	☐	☐
25. I finish the work I am doing. My attention is good	☐	☐	☐

Well done! Just a few more questions to go!

The next items ask about thoughts or beliefs that you could have had *at any time in your life*, not just in the last six months. For each item, please mark the box for Not True, Somewhat True, or Certainly True. Remember to answer all the items as best you can even if you are not absolutely certain or the item seems daft!

	Not True	Somewhat True	Certainly True
1. Some people believe that their thoughts can be read. Have other people ever read your thoughts?	☐	☐	☐
2. Have you ever believed that you were being sent special messages through the television?	☐	☐	☐
3. Have you ever thought that you were being followed or spied upon?	☐	☐	☐
4. Have you ever heard voices that other people could not hear?	☐	☐	☐
5. Have you ever felt that you were under the control of some special power?	☐	☐	☐
6. Have you ever known what another person was thinking even though that person wasn't speaking?	☐	☐	☐
7. Have you ever felt as though your body had been changed in some way that you could not understand?	☐	☐	☐
8. Do you have any special powers that other people don't have?	☐	☐	☐
9. Have you ever seen something or someone that other people could not see?	☐	☐	☐
Has this happened in the last year?	☐	☐	☐

If you have had any of the experiences described above, do these experiences upset you?	☐ No	☐ Yes
Do these experiences cause difficulties for you at home or at school?	☐ No	☐ Yes
Have you had any of these experiences during the past year?	☐ No	☐ Yes

Please tell us today's date: _____

Please tell us your birthday (day and month): _____

In which year were you born? **1994**: ☐ **1995**: ☐ **1996**: ☐ **1997**: ☐ **1998**: ☐ **1999**: ☐

Is there anything else you would like to tell us about yourself?

_____

_____

If you have any worries about the questions or your answers, and you would like to speak to a member of the research team, please contact:

Dr. Kristin Laurens (020)-7848-0964

THANK YOU VERY MUCH FOR YOUR HELP!!

Appendix 2. Caregiver screening questionnaire

Caregiver Questionnaire

This form should be filled in by the child's main caregiver (usually, this is the child's mother or father). It would help us if you answer all the questions as best you can, even if you are not absolutely certain of your answers or the questions don't seem to apply to your child.

Who is completing this form (e.g., child's Mother, Father, Grandmother, etc.)? : _____

Is your child (please mark the correct box, like this ☑): ☐ Male ☐ Female

When (date) and **Where** were these people born?:

Your child: DD / MM / YYYY City: _____ ; Country _____

Child's mother: DD / MM / YYYY City: _____ ; Country _____

Child's father: DD / MM / YYYY City: _____ ; Country _____

Did your child ever live away from London? ☐ No ☐ Yes

Which ethnic background best describes your child? (please choose **one** of the following):

White: ☐ British ☐ Irish
☐ Other White Background (specify):_____

Black or Black British: ☐ Caribbean ☐ African
☐ Other Black Background (specify): _____

Asian or Asian British: ☐ Indian ☐ Pakistani ☐ Bangladeshi
☐ Other Asian Background (specify): _____

Oriental or Oriental British: ☐ Chinese ☐ Japanese
☐ Other Oriental Background (specify): _____

Mixed: ☐ White-Black Caribbean ☐ White-Black African ☐ White-Asian
☐ Other Mixed Background (specify):_____

Other group not included above (specify):_____

If your child has any long-standing health problem or condition (e.g., diabetes, epilepsy, etc.), please tell us what it is:

_____

Has your child, or any of your child's relatives, ever seen a doctor about a mental health condition? Please tell us **who** (e.g., child, or child's brother or sister, mum or dad, grandparent, cousin, etc.) and **which condition** (e.g., stress or anxiety or nerves, depression, psychosis or schizophrenia, inattention or hyperactivity, autism, eating disorder, etc.):

_____

The next questions ask about your child's speech and motor (physical) development. Some questions ask about things that may be hard to remember. Please try to answer as accurately as you can.

At what age did your child regularly use single words (not including 'mama' or 'dada') in a meaningful way?

☐ before 1 year ☐ between 1-2 years ☐ 2-3 years ☐ 3-4 years ☐ 4 years or older ☐ don't know

At what age did your child regularly use two- and three-word phrases?

☐ before 1 year ☐ between 1-2 years ☐ 2-3 years ☐ 3-4 years ☐ 4 years or older ☐ don't know

At what age was your child able to walk on his/her own?

☐ by 6 months ☐ between 6-12 months ☐ 12-18 months ☐ 18-24 months ☐ 24 months or older ☐ don't know

In your child's first three years of life:

- Was there anything that seriously worried you about the way your child's speech developed? ☐ No ☐ Yes
- Were you ever seriously worried that your child was slow to reach his/her motor milestones (e.g., to start standing / walking)? ☐ No ☐ Yes

Did you ever speak to a professional (e.g., speech therapist, health visitor, GP, etc.) about your concerns regarding your child's speech development? ☐ No ☐ Yes

Did your health visitor, GP, or other professional ever worry that your child was late:

- To stand on his/her own? ☐ No ☐ Yes
- To walk? ☐ No ☐ Yes

Does your child have any difficulty with co-ordination or unsteadiness (e.g., during activities such as playing sport, riding a bike, dancing etc.) ☐ No ☐ Yes

The next items ask about your child's Strengths and Difficulties. For each item, please mark the box for Not True, Somewhat True, or Certainly True. It would help us if you answered all the items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of your child's behaviour *over the last six months*.

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches, or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils, etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset, or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted, or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Over the last six months, has your child had difficulties in one or more of the following areas: Emotions, concentration, behaviour, or being able to get along well with other people?

☐ No ☐ Yes - minor difficulties ☐ Yes - definite difficulties ☐ Yes - severe difficulties

If you answered "Yes", please answer the following questions about these difficulties:

- Do the difficulties upset or distress your child?

  ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

- Do the difficulties interfere with your child's everyday life in the following areas?

  Home Life: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

  Friendships: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

  Classroom Learning: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

  Leisure Activities: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

- Do the difficulties put a burden on you or the family as a whole?

  ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

The next items ask about thoughts or beliefs that your child could have had at *any time* in his/her life, not just over the last six months. For each item, please mark the box for Not True, Somewhat True, or Certainly True. Remember to answer all the items as best you can even if you are not absolutely certain or the item seems daft!

	Not True	Somewhat True	Certainly True
Some people believe that their thoughts can be read. Has your child ever thought that other people could read his/her thoughts?	☐	☐	☐
Has your child ever believed that he/she was being sent special messages through the television or the radio, or that a programme had been arranged just for him/her alone?	☐	☐	☐
Has your child ever thought that he/she was being followed or spied upon?	☐	☐	☐
Has your child ever heard voices that other people couldn't hear?	☐	☐	☐
Has your child ever thought that he/she was under the control of some special power?	☐	☐	☐
Has your child ever claimed to know what another person was thinking even though that person wasn't speaking?	☐	☐	☐
Has your child ever thought his/her body had been changed in some way that he/she couldn't understand?	☐	☐	☐
Does your child have any special powers that other people don't have?	☐	☐	☐
Has your child ever seen something or someone that other people could not see?	☐	☐	☐
Has this happened in the last year?	☐	☐	☐
Have you ever seriously worried that your child was thinking or acting in a bizarre way?	☐	☐	☐

If 'Somewhat True' or 'Certainly True', please provide details:

_____

_____

If you answered "Somewhat True" or "Certainly True" to any of the questions on this page, please tell us whether these experiences have caused any difficulties for your child **over the past year**:

☐ No ☐ Yes - minor difficulties ☐ Yes - definite difficulties ☐ Yes - severe difficulties

If you answered "Yes", please answer the following questions about these difficulties:

- Do the difficulties upset or distress your child?

  ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

- Do the difficulties interfere with your child's everyday life in the following areas?

  Home Life: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

  Friendships: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

  Classroom Learning: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

  Leisure Activities: ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

- Do the difficulties put a burden on you or the family as a whole?

  ☐ Not at all ☐ Only a little ☐ Quite a lot ☐ A great deal

Please tell us today's date: DD / MM / YYYY

If you have any concerns about the questions you have been asked or about the answers you have given, and would like to speak to a member of the research team, please contact:

Dr. Kristin Laurens (020)-7848-0964 K.Laurens@iop.kcl.ac.uk

THANK YOU VERY MUCH FOR YOUR HELP!

DON'T FORGET TO FILL IN THE CONSENT FORM IF YOU WOULD LIKE TO PARTICIPATE IN PART TWO OF THE RESEARCH

KING'S College LONDON
University of London

Appendix 3. Negative life events and daily hassles measure

Note. Negative life events and daily hassles were assessed in a single measure which included additional items not examined in this thesis.

Participant ID:
Date:
Researcher:

DHSC / PVS / NLE – Youth Form

We're going to be talking about a whole lot of different life events that young people might experience, some of which might be upsetting for you to think about. Remember, you do not have to answer any question you do not want to, and any answers you do give will be kept confidentially by the research team. If you would like to talk about anything that upsets or concerns you, please tell me.

For each question, there are two parts to answer. The first part asks you how often you have experienced the event **during the past year.** If you **have** experienced it, then please tell me also how much the experience bothered you.

During the part year how often have the following happened to you	How often does this happen to you?				How much are you bothered by this?			
	Never	Rarely	Some-times	Often	Not at all	A little	Some-what	A lot
1. You experienced difficulties with friends	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
2. Other children make negative comments about your work	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
3. Other children talk about you behind your back	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
4. Your parents give you too many jobs	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
5. A teacher lectures you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
6. The classroom is too noisy	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
7. You get detention or other punishment tasks from your teacher	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
8. You have trouble with mathematics	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
9. You have problems getting on with the other children in your class	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
10. Other children take your property or break your things	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
11. You have to sit a test	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
12. Other children swear or use foul language around you, or talk about upsetting things around you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
13. Your parents are critical of your homework	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐

1.

Please turn over the page....

		How often does this happen to you?				How much are you bothered by this?			
		Never	Rarely	Some-times	Often	Not at all	A little	Some-what	A lot
14.	Your teacher is unfair to you or to other children	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
15.	You have problems with older children	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
16.	You have problems with younger children	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
17.	A teacher makes negative comments about your work	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
18.	You have to sit next to annoying children in class	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
19.	Your parent has to work and is not at home as much as you would like	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
20.	A teacher shouts at you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
21.	You misplace or lose things	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
22.	Your parents want you to do better	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
23.	Other children don't want to be your friend	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
24.	You have too many things to do	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
25.	You forget things that need to be done	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
26.	Other children say mean or hurtful things to you, or call you mean or hurtful names	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
27.	Other children completely ignore you, or leave you out of things on purpose	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
28.	A teacher does not listen to you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
29.	You have trouble with reading, writing, or spelling	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
30.	Other children hit, kick, push, or shove you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
31.	Other children tell lies or spread rumours about you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
32.	Other children make fun of you, tease you, or pick on you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐

Please turn over the page....

		How often does this happen to you?				How much are you bothered by this?			
		Never	Rarely	Some-times	Often	Not at all	A little	Some-what	A lot
33.	You have too much homework	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
34.	Your parents check up on you with your teacher	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
35.	Your parents fight or argue	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
36.	You owe money to someone else	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
37.	Other children laugh at you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
38.	Your homework is too hard	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
39.	Other children lock you in a room or hold you down against your will	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
40.	You feel left out	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
41.	A teacher does not choose you or ignores you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
42.	Other children cut you off or interrupt you	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
43.	You don't have enough money for clothes	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
44.	You don't like the way you look	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
45.	You don't have enough time to do things you enjoy doing	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
46.	Other people owe you money	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
47.	You don't get enough sleep	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
48.	You have to visit the doctor or dentist	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
49.	You have to speak in front of the class	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐
50.	You don't have enough money for movies, video games, or activities that you enjoy	☐ ↓	☐ →	☐ →	☐ →	☐	☐	☐	☐

Please turn over the page....

This next section asks about some more specific events which may have happened at any time throughout your life, not just in the past year. These things typically happen less often than the examples that we spoke about previously and may be more upsetting to think about. If these things **have happened** to you we would like to know when or how often they have happened and how bothered you are by those things now.

		How often does this happen to you?				How much are you bothered by this?			
		Never	Rarely	Some-times	Often	Not at all	A little	Some-what	A lot
51.	You have moved home	☐ ↓	How often? _____ →			☐	☐	☐	☐
52.	You had a new baby brother or sister	☐ ↓	When? _____ →			☐	☐	☐	☐
53.	Someone very close to you died	☐ ↓	Who? _____ When? _____ →			☐	☐	☐	☐
54.	You were in a fire or natural disaster (e.g., cyclone, earthquake)	☐ ↓	What? _____ When? _____ →			☐	☐	☐	☐
55.	You had to change school	☐ ↓	When? _____ →			☐	☐	☐	☐
56.	Your parents separated or divorced	☐ ↓	When? _____ →			☐	☐	☐	☐
57.	You have been in a serious car accident	☐ ↓	When? _____ →			☐	☐	☐	☐
58.	You were seriously ill and had to go to hospital	☐ ↓	When? _____ →			☐	☐	☐	☐
59.	Your parent had a serious accident or illness	☐ ↓	When? _____ →			☐	☐	☐	☐
60.	You had to repeat a school year	☐ ↓	When? _____ →			☐	☐	☐	☐
61.	Your parent got a new boyfriend/girlfriend or remarried	☐ ↓	When? _____ →			☐	☐	☐	☐
62.	Your house was robbed	☐ ↓	When? _____ How often? _____ →			☐	☐	☐	☐
63.	You have been a victim of crime (theft, abuse, or assault)	☐ ↓	What? _____ How often? _____ →			☐	☐	☐	☐

Appendix 4. Alabama parenting questionnaire (child version)

Note. Items 33, 35, 38 used to assess physical punishment.

Participant ID:
Date:
Researcher:

APQ – Youth Form

Please read the following statements, and tick the box that shows how often the item TYPICALLY occurs in your home. If your mum or dad is not currently living at home with you, then skip the questions that ask about that person.

		Never	Almost Never	Some-times	Often	Always
1a.	You have a friendly talk with your mum	☐	☐	☐	☐	☐
	1b. How about your dad?	☐	☐	☐	☐	☐
2.	Your parents tell you that you are doing a good job	☐	☐	☐	☐	☐
3.	Your parents threaten to punish you, and then do not do it	☐	☐	☐	☐	☐
4a.	Your mum helps with some of your special activities (e.g., sports, youth groups, scouts)	☐	☐	☐	☐	☐
	4b. How about your dad?	☐	☐	☐	☐	☐
5.	Your parents reward or give something extra to you for behaving well	☐	☐	☐	☐	☐
6.	You fail to leave a note or let your parents know where you are going	☐	☐	☐	☐	☐
7a.	You play games or do other fun things with your mum	☐	☐	☐	☐	☐
	7b. How about your dad?	☐	☐	☐	☐	☐
8.	You talk your parents out of punishing you after you have done something wrong	☐	☐	☐	☐	☐
9a.	Your mum asks you about your day in school	☐	☐	☐	☐	☐
	9b. How about your dad?	☐	☐	☐	☐	☐
10.	You stay out in the evening past the time you are supposed to be home	☐	☐	☐	☐	☐
11a.	Your mum helps you with your homework	☐	☐	☐	☐	☐
	11b. How about your dad?	☐	☐	☐	☐	☐
12.	Your parents give up trying to get you to obey them because it is too much trouble	☐	☐	☐	☐	☐

		Never	Almost Never	Some-times	Often	Always
13.	Your parents compliment you when you have done something well	☐	☐	☐	☐	☐
14a.	Your mum asks you what your plans are for the coming day	☐	☐	☐	☐	☐
	14b. How about your dad?	☐	☐	☐	☐	☐
15a.	Your mum drives you to a special activity	☐	☐	☐	☐	☐
	15b. How about your dad?	☐	☐	☐	☐	☐
16.	Your parents praise you for behaving well	☐	☐	☐	☐	☐
17.	Your parents do not know the friends you are with	☐	☐	☐	☐	☐
18.	Your parents hug or kiss you when you have done something well	☐	☐	☐	☐	☐
19.	You go out without a set time to be home	☐	☐	☐	☐	☐
20a.	Your mum talks to you about your friends	☐	☐	☐	☐	☐
	20b. How about your dad?	☐	☐	☐	☐	☐
21.	You go out after dark without an adult with you	☐	☐	☐	☐	☐
22.	Your parents let you out of a punishment early (like lift restrictions earlier than they originally said)	☐	☐	☐	☐	☐
23.	You help plan family activities	☐	☐	☐	☐	☐
24.	Your parents get so busy they forget where you are and what you are doing	☐	☐	☐	☐	☐
25.	Your parents do not punish you when you have done something wrong	☐	☐	☐	☐	☐
26a.	Your mum goes to a meeting at school (like a parent-teacher meeting)	☐	☐	☐	☐	☐
	26b. How about your dad?	☐	☐	☐	☐	☐
27.	Your parents tell you they like it when you help out around the house	☐	☐	☐	☐	☐
28.	You stay out later than you are supposed to and your parents don't know it	☐	☐	☐	☐	☐

		Never	Almost Never	Some-times	Often	Always
29.	Your parents leave the house and don't tell you where they are going	☐	☐	☐	☐	☐
30.	You come home from school more than an hour past the time your parents expect you to be home	☐	☐	☐	☐	☐
31.	The punishment your parents give depends on their mood	☐	☐	☐	☐	☐
32.	You are at home without an adult being with you	☐	☐	☐	☐	☐
33.	Your parents spank you with their hand when you have done something wrong	☐	☐	☐	☐	☐
34.	Your parents ignore you when you are misbehaving	☐	☐	☐	☐	☐
35.	Your parents slap you when you have done something wrong	☐	☐	☐	☐	☐
36.	Your parents take away a privilege or money from you as a punishment	☐	☐	☐	☐	☐
37.	Your parents send you to your room as punishment	☐	☐	☐	☐	☐
38.	Your parents hit you with a belt, stick, or other object when you have done something wrong	☐	☐	☐	☐	☐
39.	Your parents yell or scream at you when you have done something wrong	☐	☐	☐	☐	☐
40.	Your parents calmly explain to you why your behaviour was wrong when you misbehave	☐	☐	☐	☐	☐
41.	Your parents use "time-out" (make you sit or stand in a corner) as a punishment	☐	☐	☐	☐	☐
42.	Your parents give you extra chores as a punishment	☐	☐	☐	☐	☐

Appendix 5. Saliva collection form

Participant ID:

SALIVA SAMPLES PARTICIPANT QUESTIONNAIRE

Because our hormone levels vary throughout the day and can be affected by what we eat, how active we are, and how we are feeling, it is very important that we collect this information from you on the days that you provide a saliva sample. Each time you collect a sample we would like you to complete the appropriate section of the questionnaire as soon as possible.

Please remember that the answers you provide on this questionnaire are completely confidential and will not be shown to anyone outside of the research team.

DAY 1

Please tell us the date today: _____

Morning samples: Samples 1, 2, 3 and 4

Please tell us the time at which you collected the following samples;

Sample 1: Awakening sample_____

Sample 2: 15 minutes after awakening sample_____

Sample 3: 30 minutes after awakening sample_____

Sample 4: 60 minutes after awakening sample_____

How you are feeling this morning

Please circle the number that best describes how you have been feeling since you woke up (for each emotion);	**Not at all**				**Very much so**
Worried or anxious	1	2	3	4	5
Happy	1	2	3	4	5
Angry or hostile	1	2	3	4	5
Depressed or sad	1	2	3	4	5
Frustrated or stressed	1	2	3	4	5

How much sleep you have had

Please tell us how many hours sleep you had LAST NIGHT; _____
Please tell us how many hours you USUALLY sleep for on an average night;_____

Additional information	**No**	**Yes**
Have you consumed any alcoholic drinks in the past 24 hours?	☐	☐
If yes, please tell how when you last had a drink containing alcohol; _____		
Have you smoked any cigarettes since waking up this morning?	☐	☐
If yes, please tell us how many cigarettes you have smoked;_____		

Participant ID:

SALIVA COLLECTING PARTICIPANT QUESTIONNAIRE

DAY 1

Sample 5; 12 pm (midday)

Please tell us the exact time at which you collected this sample_____

Please tell us what you were doing before collecting this sample (for example; watching television, playing on the computer, eating etc.)

_____
_____
_____

Please tell us what you have had to eat and drink since getting up (we are particularly interested in whether you have had any food or drink containing dairy, e.g., milk, cheese or yoghurt).

_____
_____
_____

Time food and/or drink consumed_____

How you are feeling this morning

Please circle the number that best describes how you have been feeling this morning (for each emotion);	**Not at all**				**Very much so**
Worried or anxious	1	2	3	4	5
Happy	1	2	3	4	5
Angry or hostile	1	2	3	4	5
Depressed or sad	1	2	3	4	5
Frustrated or stressed	1	2	3	4	5

Additional information	**No**	**Yes**
Have you consumed any alcoholic drinks in the last hour	☐	☐
If yes, please tell us how many alcoholic drinks you have had in the last hour; _____		
Have you smoked any cigarettes since in the past hour?	☐	☐
If yes, please tell us how many cigarettes you have smoked in the last hour;_____		

Participant ID:

SALIVA SAMPLES PARTICIPANT QUESTIONNAIRE

DAY 1

Sample 6; 8 pm

Please tell us the exact time at which you collected this sample_____

Please tell us what you were doing before collecting this sample (for example; watching television, playing on the computer, eating etc.)

_____
_____
_____

Please tell us what you have had to eat and drink this afternoon/evening (we are particularly interested in whether you have had any food or drink containing dairy, e.g., milk, cheese or yoghurt).

_____
_____
_____

Time food and/or drink consumed_____

How you are feeling this morning

Please circle the number that best describes how you have been feeling this morning (for each emotion);	**Not at all**				**Very much so**
Worried or anxious	1	2	3	4	5
Happy	1	2	3	4	5
Angry or hostile	1	2	3	4	5
Depressed or sad	1	2	3	4	5
Frustrated or stressed	1	2	3	4	5

Additional information	**No**	**Yes**
Have you consumed any alcoholic drinks in the last hour	☐	☐
If yes, please tell us how many alcoholic drinks you have had in the last hour; _____		
Have you smoked any cigarettes since in the past hour?	☐	☐
If yes, please tell us how many cigarettes you have smoked in the last hour;_____		